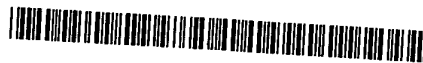


(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/36685 A2

(51) International Patent Classification⁷: **C12Q 1/68**

(21) International Application Number: **PCT/US00/31809**

(22) International Filing Date:
17 November 2000 (17.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/166,056 17 November 1999 (17.11.1999) US
60/166,106 17 November 1999 (17.11.1999) US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 01/36685 A2

(54) Title: **DIFFERENTIAL GENE EXPRESSION IN CANCER**

(57) Abstract: The invention is directed towards methods for ascertaining gene expression characteristic for cancer, in particular brain cancers such as glioblastoma, and the sequences identified thereby. Compositions, methods and kits encompassing such are provided herein.

DIFFERENTIAL GENE EXPRESSION IN CANCER

Field of the Invention

The invention relates to the field of cancer, in particular characteristic genes and gene expression useful in screening for, diagnosis of, monitoring of, and therapeutic treatment of cancer. Further, the invention relates to age-related differential expression of genes in cancer.

Background of the Invention

Cancer can develop in any tissue of any organ at any age. Most cancers detected at an early stage are potentially curable; thus, physicians need a heightened awareness of predisposing inherited and environmental factors. The ability to screen patients for genetic predisposition for cancer can greatly assist in the monitoring of high-risk patients for early signs of cancer, and thus allowing for early intervention. (See for example, The Merck Manual of Diagnosis and Therapy, 16th ed., Merck & Co., (1992)).

Malignant brain tumors (for example glioma, meningiomas, and schwannomas) are common, with an incidence of 4.5 per 100,000. The most common tumor types in adults are gliomas and meningiomas. The most common tumors in children are astrocytomas, medulloblastomas, ependymomas, and brain stem gliomas. In children, brain tumors are one of the most common causes of death from cancer. (See for example, Professional Guide to Disease, 3rd ed., Springhouse Corp., (1989)).

Clinically, brain tumors can be characterized by their cell type and location, along with other phenotypic clues. Malignant brain tumors are sometimes catagorized as glioblastoma multiforme (spongioblastoma multiforme), astrocytoma, oligodendroglioma, ependyoma, medulloblastoma, meningioma, schwannoma, and pituitary tumors. It is also possible that cancer originating in other tissues, such as lung, liver, pancreas, colon, prostate etc., can metastasize to the brain, thus forming tumors that are not of brain origin, potentially causing confusion as to the source of cancer.

Cancer is a cellular malignancy whose unique trait - loss of normal control mechanisms - results in unregulated growth, lack of differentiation, and ability to invade local tissues and metastasize. Thus cancer cells are unlike normal cells, and are potentially identifiable by not only their phenotypic traits, but also by their biochemical and molecular biological characteristics. In particular, the altered phenotype of cancer cells indicates altered gene activity, either unusual

gene expression, or gene regulation. Identification of gene expression products or proteins associated with cancer cells will allow for the molecular characterization of malignancies. The ability to specifically characterize suspected cancers, and to potentially identify not only cell type, but also predisposition for metastasis and any sensitivity to particular anti-cancer therapy, is most useful for determining not only the course of treatment, but also the likelihood of success.

Thus, the discovery of specific, brain tumor characteristic gene expression is a useful and important tool useful in screening for, diagnosis of, monitoring of, and therapeutic treatment of brain cancer. In particular, provided herein are methodologies and sequences that are differentially expressed in cancer from age-differentiated patients.

Summary of the Invention

The identification of characteristic, nucleic acid signals is a useful and important discovery which allows for compositions, assays, kits and reagents suitable for the characterization of various brain cancers. Provided herein are reagents and methods for ascertaining the propensity of a cell for malignant phenotype said cell being isolated or in a biological sample, said method comprising assaying a cell or biological sample to be tested for a signal indicating the transcription of a nucleic acid transcript. In a preferred embodiment, the nucleic acids are substantially identical to the sequences of SEQ ID NOS. 1-184, or fragments thereof. Also provided are methods for monitoring cancer progression or the effectiveness of a treatment regimen, and methods for identifying compounds that affect expression of genes involved in cancer.

One of ordinary skill in the art will be able to understand and ascertain modifications and embodiments of the present invention that fall within the spirit and scope of the disclosure as described below.

Brief Description of the Figures

Figure 1. Relationship between patient age at diagnosis and glioma survival

The survival pattern for Grade IV astrocytoma (GBM) patients according to four age strata is illustrated. The apparent differences between the <35 and the 35-50 year group are not statistically significant, but the survival for the <50 year group as a whole was statistically different from the 50-65 and the >65 year groups (Wilcoxon test, $p=.002$).

Figure 2. Northern analysis of hsp60 mRNA. 25 µg of total RNA was isolated, electrophoresed through 1.2% agarose-formaldehyde gels, transferred to nylon membranes and hybridized with a uniformly (³²P)-labeled hsp60-specific cDNA probe. (A): hsp60 expression in normal (NL) brain and GBMs. Patient age at diagnosis is depicted. (B): Developmental expression of hsp60 in normal brain tissue.

Figure 3. Normal Developmental Expression of Heat Shock Proteins in Human Brain. 25 µg of total RNA was isolated, electrophoresed through 1.2% agarose-formaldehyde gels, transferred to nylon membranes and hybridized with a uniformly (³²P)-labeled cDNA probes specific for hsp27, hsp70, hsc72, hsp90α, hsp90β, and GRP78.

Figure 4. Differential Expression of Heat Shock Proteins in Human Gliomas 25 µg of total RNA was isolated, electrophoresed through 1.2% agarose-formaldehyde gels, transferred to nylon membranes and hybridized with a uniformly (³²P)-labeled cDNA probes specific for hsp27, hsp70, hsc72, and hsp90β.

15 Detailed Description of the Invention

It is believed that brain tumorigenesis results from complex interactions of multiple and cumulative genetic alterations. These events lead to either the activation of various oncogenes, overriding regulatory signals which control cell proliferation, or inactivation of tumor suppressor genes, resulting in the uncontrolled growth of cells. (See for example Burck et al., *Oncogenes*, Springer-Verlag, New York, 1988). The identification and characterization of subsets of the genes associated with such uncontrolled growth is essential in order to understand the process of malignancy, but more importantly, useful for the identification of specific cancerous tissues, and tissues that are premalignant, and potentially predisposed for it.

Cancer is defined herein as any cellular malignancy for which a loss of normal cellular controls results in unregulated growth, lack of differentiation, and increased ability to invade local tissues and metastasize. Cancer may develop in any tissue of any organ at any age. Cancer may be an inherited disorder or caused by environmental factors or infectious agents; it may also result from a combination of these.

The differential expression of genes that regulate cell growth, migration, and other functions enables a cell to grow out of control and become cancerous. In many cases, the

activation of oncogenes, which override the intrinsic cellular growth regulatory commands of a cell, as well as the inactivation of tumor suppressor genes, which normally hold tumor formation in check, renders tumor cells free of growth restraints. The identification and characterization of these differentially expressed genes in malignant tumors will facilitate the understanding of the basic nature of the malignancy and yield novel molecular markers useful in diagnosis and treatment. For the purposes of utilizing the present invention, the term cancer includes both neoplasms and premalignant cells.

In one embodiment, the present invention is useful for the diagnosis and treatment of many types of cancers including, for example, cancers of the breast, prostate, colon, and lung.

In a preferred embodiment, the reagents and methodologies provided herein are useful for the diagnosis and treatment of brain cancer. Brain tumors (or brain cancer) arise as a result of complex interactions of multiple and cumulative genetic alterations. Brain cancer is defined herein as any cancer involving a cell of neural origin. Examples of brain cancers include but are not limited to intracranial neoplasms such as those of the skull (i.e., osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), the meninges (i.e., meningioma, sarcoma, gliomatosis), the cranial nerves (i.e., glioma of the optic nerve, schwannoma), the neuroglia (i.e., gliomas) and ependyma (i.e., ependymomas), the pituitary or pineal body (i.e., pituitary adenoma, pinealoma), and those of congenital origin (i.e., craniopharygioma, chordoma, germinoma, teratoma, dermoid cyst, angioma, hemangioblastoma) as well as those of metastatic origin.

As demonstrated herein, it has been discovered that brain cancer cells, in particular glioma cells, express certain nucleic acid sequences at a higher level than that found in normal brain cells, for example fetal astrocytes. Similarly, it has been found that this expression is most commonly detected as a nucleic acid, usually mRNA which is expressed from an activated gene, resulting in a detectable nucleic acid signal corresponding to the transcript from a gene. The present invention teaches a specific array of gene signals, i.e. expressed genes, mRNA transcripts, which indicate a cells propensity for a malignant phenotype in cancer. In a preferred embodiment, the gene sequences provided herein are indicative of brain cancer. In addition, the present invention provides an assay system for the detection of cancer and the monitoring of treatment progress. In one embodiment, a panel comprising one or more of SEQ ID NOS. 1-141, or fragments or complements thereof, may be utilized to identify cancerous cells. In a preferred

embodiment, the panel comprises one or more of SEQ ID NOS. 68, 69 or 183, or fragments or complements thereof.

One of the most significant factors impacting the survival of patients with glioblastomas (GBM) is age at primary diagnosis. Patients diagnosed prior to the age of 50 years survive significantly longer than those diagnosed after the age of 50, with median survival of 24 months and 8 months, respectively. This difference in survival is independent of performance status and appears to be unrelated to treatment. The cellular mechanisms for this age/prognosis correlation are not known. Several age-related chromosomal aberrations in GBM have been recently described, and include +7, amplifications on 7, -18q and -10 in tumors from older patients. Additionally, +17q, -Xp, -5q, and -10q have been found to occur in tumors from younger patients. These data strongly suggests a molecular basis for this poor patient survival. Provided herein is a DDRT-PCR based approach to define molecular changes associated with this age-dependent survival of GBM patients, and a panel of differentially expressed genes from tumors resected from these disparate patient populations. The present invention further provides novel nucleic acid sequences representing genes and the polypeptides encoded thereby that are involved in cancer progression.

In one embodiment, the expression of a panel of sequences comprising one or more of SEQ ID NOS. 142-182, or fragments or complements thereof, may be assayed to characterize the tumors of old vs. young patients. In a preferred embodiment, the panel comprises one or more of SEQ ID NOS. 142-174, or fragments of complements thereof, where over-expression in tumors of old patients as compared to young patients of the sequences is detected. In a more preferred embodiment, the panel comprises one or more of SEQ ID NOS. 142, 143, 144, 147, 149, 162 or 173, or fragments or complements thereof, where increased expression of the sequences in tumors of old patients as compared to young patients is detected. In another preferred embodiment, the panel comprises one or more of SEQ ID NOS. 175-182, or fragments of complements thereof, where decreased expression of the sequences in tumors of old patients as compared to young patients is detected.

I. General Methodology

Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references including: *Molecular Cloning: A Laboratory Manual*

- (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), Berger et al., Guide to Molecular Cloning Techniques, *Methods in Enzymology*, Vol. 152, Academic Press, Inc., (1987); Davis et al., *Basic Methods in Molecular Biology*, Elsevier Science Publishing Co., Inc. (1986); Ausubel et al., *Short Protocols in Molecular Biology*, 2nd ed., John Wiley & Sons, (1992), Grinsted et al., *Plasmid Technology*, *Methods in Microbiology*, Vol. 21, Academic Press, Inc., (1988); Symonds et al., *Phage Mu*, Cold Spring Harbor Laboratory Press (1987), Guthrie et al., Guide to Yeast Genetics and Molecular Biology, *Methods in Enzymology*, Vol. 194, Academic Press, Inc., (1991), *PCR Protocols: A Guide to Methods and Applications* 10 (Innis, et al. 1990. Academic Press, San Diego, CA), McPherson et al., *PCR Volume 1*, Oxford University Press, (1991), *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Ed. (R.I. Freshney. 1987. Liss, Inc. New York, NY), and *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.). The basic principles of eukaryotic gene structure and expression are generally known in the art. (See for example 15 Hawkins, *Gene Structure and Expression*, Cambridge University Press, Cambridge, UK, 1985; Alberts et al., *The Molecular Biology of the Cell*, Garland Press, New York, 1983; Goeddel, *Gene Expression Technology*, *Methods in Enzymology*, Vol. 185, Academic Press, Inc., (1991); Lewin, *Genes VI*, Oxford Press, Oxford, UK, 1998). Each of the above-mentioned references and any of those listed below including issued patents are hereby incorporated by reference.

20 For the purposes of this application, certain terms are defined below. The meaning of these terms are generally understood by those of skill in the art, and the descriptions provided herein are provided merely as additional guidance.

A *transcriptional regulatory region* is defined as any region of a gene involved in regulating transcription of a gene, including but not limited to promoters, enhancers and 25 repressors. A *transcriptional regulatory element* is defined as any element involved in regulating transcription of a gene, including but not limited to promoters, enhancers and repressors. A *promoter* is a regulatory sequence of DNA that is involved in the binding of RNA polymerase to initiate transcription of a gene. A *gene* is a segment of DNA involved in producing a peptide, polypeptide or protein, including the coding region, non-coding regions preceding ("leader") and 30 following ("trailer") the coding region, as well as intervening non-coding sequences ("introns") between individual coding segments ("exons"). *Coding* refers to the representation by the nucleic

acid of amino acids, start and stop signals in a three base "triplet" code. Promoters are often upstream ("5' to") the transcription initiation site of the corresponding gene. Other regulatory sequences of DNA in addition to promoters are known, including sequences involved with the binding of transcription factors, including response elements that are the DNA sequences bound by inducible factors. *Enhancers* comprise yet another group of regulatory sequences of DNA that can increase the utilization of promoters, and can function in either orientation (5'-3' or 3'-5') and in any location (upstream or downstream) relative to the promoter. Preferably, the regulatory sequence has a positive activity, i.e., binding of an endogeneous ligand (e.g. a transcription factor) to the regulatory sequence increases transcription, thereby resulting in increased expression of the corresponding target gene. The term *operably linked* refers to the combination of a first nucleic acid fragment representing a transcriptional control region having activity in a cell joined to a second nucleic acid fragment encoding a reporter or effector gene such that expression of said reporter or effector gene is influenced by the presence of said transcriptional control region.

15 A *polypeptide* refers to an amino acid sequence encoded by a nucleic acid, a fragment thereof, or a nucleic acid comprising a nucleic acid of this invention. Preferably, the nucleic acids of this invention are selected from those described by SEQ ID NOS. 1-184.

20 A *nucleic acid* or *protein fragment* relates to a portion of a larger sequence from which the fragment is derived, where the fragment is useful for performing the methods described herein. For instance, a particular sequence described within this application may contain irrelevant nucleotides derived from a cloning vector or primer used in amplifying the nucleic acid (i.e., HindIII site, poly-A, poly-T). Those nucleotides could be deleted from the particular sequence, resulting in a functional fragment of the larger sequence. Similarly, a portion of a sequence (i.e., 15 nucleotides of a 200 bp nucleic acid) may be utilized for detecting expression of a gene sequence within a cell. A protein fragment is a sequence of amino acids derived from a protein that is functional, as an immunogen, a probe to detect autoantibodies, or to identify relevant ligands, for example.

25 A *responsive element* is a portion of a transcriptional control region that induces expression of a nucleotide sequence following the interaction of a cell with a compound. There may be multiple responsive elements within a single transcriptional control region and each of these elements may function independently of any other elements of that transcriptional control

30

region. Thus, a responsive element may be incorporated into a reporter gene vector independent from the remainder of the transcriptional control region from which it is derived and function to drive expression of the reporter gene under the proper conditions.

The terms *overexpressed* or *underexpressed* typically relate to expression of a nucleic acid sequence or protein in a tumor cell at a higher or lower level, respectively, than that level typically observed in a non-tumor cell (i.e., normal control). For instance, a particular sequence may be over- or under-expressed in cells or tissue obtained from a patient older than 60 years ("old" patient) as compared to a sample of cells or tissue obtained from a patient younger than 45 years old ("young" patient). In certain cases, the terms *overexpressed* or *underexpressed* may also relate to the expression level in a cell that has been contacted by a compound and compared to the expression level in a similar cell that has not been contacted by the compound.

The terms *cancer cell* and *tumor cell* and the like may be used interchangeably and relate to cells found within a cancerous growth or tumor. The reagents and methodologies provided herein are applicable to the detection, diagnosis, and treatment of many types of cancers. In a preferred embodiment, the reagents and methodologies provided herein are useful for the detection, diagnosis, and treatment of brain cancer.

For the purposes of this application, hybridization is typically performed under stringent conditions. The term *stringent conditions* refers to hybridization and washing under conditions that permit only binding of a nucleic acid molecule such as an oligonucleotide or cDNA molecule probe to highly homologous sequences. For example, a stringent wash solution is 0.015 M NaCl, 0.005 M NaCitrate, and 0.1% SDS used at a temperature of 55°C-65°C. Another stringent wash solution is 0.2X SSC and 0.1% SDS used at a temperature of between 50°C-65° C.

A nucleic acid, DNA, RNA or amino acid sequence is *identical* or the *same* as another sequence where the sequences are identical. A nucleic acid, DNA, RNA or amino acid sequence is *substantially identical* or *substantially the same* as another sequence where the sequences are 50-100% identical. In a preferred embodiment, substantially identical sequences share 60-100% identity, more preferably 70-100% identity, even more preferably 80-100% identity and even more preferably 90-100% identity. In a most preferred embodiment, substantially identical sequences share 95-100% identity. A substantially identical sequence may also relate to a complementary sequence.

Within the sequences of this application, symbols are utilized to identify those

nucleotides that may be represented by more than one of A, T, G, or C. As such, "N" denotes any of A, C, G or T; "R" denotes A or G (purine); "Y" denotes G or T (keto); "M" denotes G or C; and, "W" denotes A or T.

5 The term *antibody* in its various grammatical forms is used herein to refer to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antibody combining site or paratope. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and portions of an immunoglobulin molecule, including those portions known in the art as Fab, Fab', F(ab')₂ and F(v).

10 The word *inoculum* in its various grammatical forms is used herein to describe a composition containing a polypeptide of this invention as an active ingredient used for the preparation of antibodies against the polypeptide. When a polypeptide is used in an inoculum to induce antibodies it is to be understood that the polypeptide can be used in various embodiments, e.g., alone or linked to a carrier as a conjugate, or as a polypeptide polymer.

15 However, for ease of expression and in context of a polypeptide inoculum, the various embodiments of the polypeptides of this invention are collectively referred to herein by the term polypeptide and its various grammatical forms.

II. Detection of Nucleic Acids

20 In one embodiment, the present invention provides for the detection of gene expression where said detected signal is detected as a polynucleotide (such as an RNA, mRNA, DNA, cDNA, or other nucleic acid) or a protein / polypeptide. It should be understood by the skilled artisan that many methods for detection of such signals exist and that any suitable method for detection is encompassed by the instant invention. Typical assay formats utilizing nucleic acid

25 hybridization includes, and are not limited to, 1) nuclear run-on assay, 2) slot blot assay, 3) northern blot assay (Alwine, et al. Proc. Natl. Acad. Sci. 74:5350), 4) magnetic particle separation, 5) Nucleic Acid or DNA chips, 6) reverse northern blot assay, 7) dot blot assay, 8) *in situ* hybridization, 9) RNase protection assay (Melton, et al. Nuc. Acids Res. 12:7035 and as described in the 1998 catalog of Ambion, Inc., Austin, TX), 10) ligase chain reaction, 11)

30 polymerase chain reaction (PCR), 12) reverse transcriptase (RT)-PCR (Berchtold, et al. Nuc. Acids. Res. 17:453), and, 13) differential display RT-PCR (DDRT-PCR) or other suitable

combination of techniques and assays. Methods for detection which can be employed include, and are not limited to 1) radioactive labels, 2) enzyme labels, 3) chemiluminescent labels, 4) fluorescent labels, or other suitable labels. Such methodologies and labels, as well as many other suitable techniques not listed here, are well known in the art and widely available to the skilled
5 artisan.

In an exemplary embodiment, the RNase protection assay may be utilized in the present invention by hybridizing multiple DNA probes corresponding to a one or more members of a panel of sequences to mRNA isolated from a tumor cell and performing the RNase assay. An increase or a decrease in the expression of the sequences from the tumor cell as compared to
10 normal cells indicates that the genes related to those sequences may be involved in tumorigenesis. In a preferred embodiment, the panel is selected from the sequences shown in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

In another embodiment, multiple DNA probes capable of hybridizing to mRNA corresponding to a reporter sequence under the transcriptional control of a nucleic acid sequence
15 under- or overexpressed in tumor cells transcriptional control region may be utilized. Exemplary reporter sequences may include β -galactosidase, luciferase, CAT, and green fluorescent protein. An increase or a decrease in the expression of the sequences from the tumor cell as compared to normal cells indicates that the genes related to those sequences may be involved in tumorigenesis. In a preferred embodiment, the panel is selected from the sequences of SEQ ID
20 NOS. 1-184, sequences complementary thereto, or fragments thereof.

The screening assays of the present invention are also well suited for polymerase chain reaction (PCR) amplification, whether the format of such assays are in solution after isolation of mRNA and subsequent direct amplification or such after reverse transcription. Such assays can be performed on isolated biological samples or extracted fluids, using a suitable PCR assay
25 format. The screening methods and compositions of the present invention are also amendable to routine adaptation to automated screening systems employing computer controlled reagent aliquoting and signal detection.

With a known gene target, it is possible to apply standard PCR to assay tissue for specific gene expression (Mok et al., (1994), Gynecologic Oncology, 52: 247-252). However, detection
30 of unknown gene expression requires additional manipulations before a useful gene can be identified. Differential Display Reverse Transcriptase Polymerase Chain Reaction (DDRT-PCR)

is a powerful tool useful for isolating large numbers of expressed nucleic acids, corresponding to gene expression. Several U.S. Patents have been issued relating to methods in this and related methods, including U.S. Patents number 5,599,672; 5,807,680; 5,459,037; 5,814,445; 5,104,792; 4,683,195; 5,665,547; 5,262,311; 5,599,696; and, 5,712,126, to name a few (all of which are hereby incorporated by reference in their entirety). DDRT-PCR has been described by Liang and Pardee (Science, 1993, 257: 967-971); Liang et al. (Nucleic Acids Research, 1993, 21(14): 3269-3275); and, Wang et al. (Trends in Pharmacological Science, 1996, 17(8): 276-9).

Previous attempts to assay brain tumors include the studies of Uchiyama et al. (Neurosurgery, 1995, 37(3): 464-469); Sehgal et al. (J. of Surgical Oncology, 1997, 64: 102-108); Sehgal et al. (Int. J. Cancer, 1997, 71: 565-572); Shinoura et al. (Cancer Letters, 1995, 89: 215-221); and Kito et al. (Gene, 1997, 184: 73-81). However, the direct application of DDRT-PCR to brain tumor samples results in a large number of signals corresponding to expressed genes, not all of which are useful for characterizing the cancerous nature of the brain tumor. Selection of the most significant signals from the large number of signals initially generated, and the assembly of a panel of characteristic nucleic acid targets requires insightful consideration and comparison of the data, followed by re-analysis and assessment of the correctness of such choices. The instant invention provides such a method for the identification of over- or under-expressed sequences in cancer. Preferably, the cancer is of neural origin.

Once identified, the specific nucleic acid targets identified as being characteristic for brain cancer can be readily adapted to automated detection assays for use in diagnosis or screening of patients for predisposition for brain cancer. Modification of the discovery of the unique panel of signals of the present invention for use in such screening or diagnostic assays would be well within the skill of one of ordinary art, and require only routine experimentation.

In one embodiment, detection of a nucleic acid such as an mRNA may be accomplished using a gene chip. For instance, the sequences of interest may be arrayed upon a chip as described in any of the available gene chip technologies such as that described by Schena, et al. (*Parallel human genome analysis: microarray-based expression monitoring of 1000 genes*. Proc Natl Acad Sci USA, 1996 Oct 1;93(20):10614-9). In that study, DNA "chips" were used to quantitatively monitor differential expression of heat shock and phorbol ester-regulated genes in human T cells. Heller, et al. (*Discovery and analysis of inflammatory disease-related genes using cDNA microarrays*. Proc Natl Acad Sci USA, 1997, Mar 18;94(6):2150-5) used DNA

chips to profile expression of selected human genes of probable significance in inflammation as well as with genes expressed in peripheral human blood cells. In that study, mRNA from cultured macrophages, chondrocyte cell lines, primary chondrocytes, and synoviocytes provided expression profiles for selected cytokines, chemokines, DNA binding proteins, and matrix-degrading metalloproteinases. From the peripheral blood library, tissue inhibitor of metalloproteinase 1, ferritin light chain, and manganese superoxide dismutase genes were identified as expressed differentially in rheumatoid arthritis compared with inflammatory bowel disease. Several other methods for utilizing DNA chips are known, including the methods described in U.S. Patents 5,744,305; 5,733,729; 5,710,000; 5,631,734; 5,599,695; 5,593,839; 5,578,832; 5,556,752; 5,770,722; 5,770,456; 5,753,788; 5,688,648; 5,753,439; 5,744,306 (all of which are incorporated by reference in their entirety).

Adaptation of the teachings of the present invention for nucleic acid or gene chip technology as described above would be routine, following the methods and teachings known in the art. The instant invention provides a DNA chip comprising specific sequences for measuring expression levels of certain sequences within a cancer cell to determine whether expression is up- or down-regulated. For instance, a DNA chip comprising nucleotide sequences capable of hybridizing to one or more members of a panel of DNA sequences may be synthesized using commonly available techniques. mRNA is isolated from a normal, non-cancer cell and a cancer cell and hybridized to the DNA chip comprising one of more of the sequences from the panel. Hybridization is then detected by any of the available methods. In such a manner, sequences that are either overexpressed or underexpressed in a cancer cell as compared to a normal cell are. In a similar manner, mRNA from a cancer cell that has been contacted with a compound may be hybridized to sequences on the DNA chip to determine whether that compound affects expression of a particular sequence. The appropriate controls should be included such that a true comparison can be made. In a preferred embodiment, the members of the panel are selected from the sequences shown in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

The invention provides for a kit comprising hybridization probes specific for at least two nucleic acid sequences selected from the group consisting of the characteristic nucleic acid sequences that are over- or under-expressed in a cancer cell. Preferably, the sequences are substantially identical to those identified in SEQ ID NOS. 1-184, sequences complementary

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thereto, or fragments thereof. In a preferred embodiment, the invention encompasses screening assays for the detection of the expression of at least one of the characteristic nucleic acid sequences identified in SEQ ID NOS. 1-184 below for the diagnosis of potentially cancerous tissues or cells. The invention provides for such a kit, further comprising suitable reaction buffer components. The invention also provides for such a kit wherein said probes are suitable for use in PCR amplification of the specific target, direct or indirect hybridization assay, RNase protection assay. In particular, such screening assays can be performed on tissue biopsy samples, serum samples, cerebro-spinal fluid samples, or any other suitable biological sample.

In another embodiment of the invention, genomic screening assays are contemplated for the detection of specific single nucleotide polymorphisms (SNP) in a nucleic acid sequence found to be over- or under-expressed in a cancer cell. Preferably, the sequence is substantially identical to those listed in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. In a preferred embodiment, such genomic screening is used to detect any predisposition for cancer formation, as an aid to assist monitoring for potential cancer episodes in the future.

Screening assays for detection of at least one of nucleic acids found to be over- or under-expressed in a cancer cell can be designed on the basis of specific hybridization, under stringent conditions, of at least one probe encompassing a specific nucleic acid sequence. Preferably, the sequence is substantially identical to those of SEQ ID NOS. 1-184, a fragment of such nucleic acid sequence, or as the assay format may require, the complementary nucleic acid sequence, or fragment thereof. The assay can be designed to detect a single species of nucleic acid that is substantially identical to the sequences of SEQ ID NOS. 1-184 in a single assay, or using the properly distinguishable signal mechanisms, more than one specific species per reaction.

In particular, the present invention teaches that the presence of detectable nucleic acid signal corresponding to the nucleic acid sequence of the cDNAs comprising the nucleic acid sequence of one or more of the sequences of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. Thus it is a further aspect of the present invention that the detection of nucleic acid corresponding to novel human genes containing the nucleic acid sequence of one or more of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof as indicative of cancer potential.

III. Methods for Cloning

The identification and isolation of the full-length genes associated with the nucleic acids that found to be over- or under-expressed in a cancer cell provides for the generation of recombinant proteins, via recombinant DNA methodologies, which can be used in numerous ways to prepare and screen for therapeutics that will interact with the protein, such as antibodies and chemical agents. Preferably, the sequence is substantially identical to a sequence of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

A full length polypeptide or fragment thereof encoded by a nucleic acid of the instant invention can be prepared using well known recombinant DNA technology methods such as those set forth in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)) and/or Ausubel et al., eds, (Current Protocols in Molecular Biology, Green Publishers Inc. and Wiley and Sons, N.Y. (1994)). A gene or cDNA encoding protein or fragment thereof may be obtained for example by screening a genomic or cDNA library, or by PCR amplification. Improved methods of cloning *in vitro* amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039.

For screening, the probe preferably has a nucleotide sequence corresponding to, complementary to, or substantially identical to a sequence over- or under-expressed in a cancer cell, preferably being a sequences substantially identical to a sequence of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. To probe a cDNA or genomic library using an oligonucleotide probe, the following exemplary hybridization conditions may be utilized: 6X.SSC with 0.05 percent sodium pyrophosphate at a temperature of 35°C-62°C, depending on the length of the oligonucleotide probe. For example, 14 base pair probes may be washed at 35-40°C, 17 base pair probes may be washed at 45-50°C., 20 base pair probes may be washed at 52-57°C, and 23 base pair probes may be washed at 57-63°C. The temperature can be increased 2-3°C where the background non-specific binding appears high. Another exemplary protocol uses tetramethylammonium chloride (TMAC) for the washing step. An exemplary stringent washing solution is 3 M TMAC, 50 mM Tris-HCl, pH 8.0, and 0.2% SDS. As described above, the washing temperature using this solution is a function of the length of the probe (ie, a 17 base pair probe is washed at about 45-50°C).

Alternatively, a gene encoding the polypeptide or fragment may be prepared by chemical synthesis using methods well known to the skilled artisan such as those described by Engels, et

al. (Angew. Chem. Intl. Ed., 28:716-734 (1989)). These methods include, inter alia, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the polypeptide, depending on whether the polypeptide produced in the host cell is secreted from that cell.

The gene or cDNA so isolated can be inserted into an appropriate expression vector for expression in a host cell. The vector is typically selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). The polypeptide or fragment thereof may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend at least in part on whether the polypeptide or fragment thereof is to be glycosylated and/or phosphorylated. If so, yeast, insect, or mammalian host cells are preferable; yeast cells can typically glycosylate and phosphorylate the polypeptide, and insect and mammalian cells can glycosylate and/or phosphorylate the polypeptide as it naturally occurs on the TRIP1 polypeptide (i.e., "native" glycosylation and/or phosphorylation).

Typically, the vectors used in any of the host cells will contain 5' flanking sequence (also referred to as a "promoter") and other regulatory elements as well such as an enhancer(s), an origin of replication element, a transcriptional termination element, a complete intron sequence containing a donor and acceptor splice site, a signal peptide sequence, a ribosome binding site element, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element.

IV. Methods for Detection of Polypeptides

The invention provides for a method wherein a protein encoded by said expressed gene is detected by protein gel assay, antibody binding assay, or other such detection as is known in the art. For instance, the present invention contemplates a kit comprising specific probes for

detection of a polypeptide product (or fragment thereof) of a sequence that is over- or underexpressed in a cancer cell where such probe can be functionalized antibody protein, polyclonal antibody, monoclonal antibody, or antigen binding fragment of such proteins. Preferably, the nucleic acid encoding the polypeptide or fragment thereof is substantially identical to a sequence of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

An antibody of the present invention, in one embodiment, is characterized as comprising antibody molecules that immunoreact with a protein encoded by a nucleic acid over- or underexpressed in cancer. Preferably, the nucleic acid is substantially identical to a sequence of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. Preferably, an antibody further immunoreacts with the protein in situ, i.e., in a tissue section. Thus, the invention describes an anti-protein antibody that immunoreacts with any of the polypeptides of this invention, preferably also immunoreacts with the recombinant protein corresponding to a nucleic acid of the instant invention, and more preferably also reacts with a native protein in situ in a tissue section.

An antibody of the present invention is typically produced by immunizing a mammal with an inoculum containing a polypeptide of this invention and thereby induce in the mammal antibody molecules having immunospecificity for immunizing polypeptide. The antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, by using DEAE Sephadex or Protein G to obtain the IgG fraction.

Exemplary antibody molecules for use in the diagnostic methods and systems of the present invention are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab', F(ab')₂ and F(v). Fab and F(ab')₂ portions of antibodies are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibodies by methods that are well known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous and Dixon. Fab' antibody portions are also well known and are produced from F(ab')₂ portions followed by reduction of the disulfide bonds linking the two heavy reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent

such as iodoacetamide. An antibody containing intact antibody molecules are preferred, and are utilized as illustrative herein.

The preparation of antibodies against polypeptide is well known in the art. See Staudt et al., J. Exp. Med., 157:687-704 (1983), or the teachings of Sutcliffe, J.G., as described in United States Patent No. 4,900,811, the teaching of which are hereby incorporated by reference. Briefly, to produce a peptide antibody composition of this invention, a laboratory mammal is inoculated with an immunologically effective amount of a polypeptide of this invention typically as present in a vaccine of the present invention. The anti-polypeptide antibody molecules thereby induced are then collected from the mammal and those immunospecific for both a polypeptide and the corresponding recombinant protein are isolated to the extent desired by well known techniques such as, for example, by immunoaffinity chromatography.

To enhance the specificity of the antibody, the antibodies are preferably purified by immunoaffinity chromatography using solid phase-affixed immunizing polypeptide. The antibody is contacted with the solid phase-affixed immunizing polypeptide for a period of time sufficient for the polypeptide to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

For a polypeptide that contains fewer than about 35 amino acid residues, it is preferable to use the peptide bound to a carrier for the purpose of inducing the production of antibodies. One or more additional amino acid residues can be added to the amino- or carboxy-termini of the polypeptide to assist in binding the polypeptide to a carrier. Cysteine residues added at the amino- or carboxy-termini of the polypeptide have been found to be particularly useful for forming conjugates via disulfide bonds. However, other methods well known in the art for preparing conjugates can also be used. The techniques of polypeptide conjugation or coupling through activated functional groups presently known in the art are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8, Suppl. 7:7-23 (1978) and U.S. Patent No. 4,493,795, No. 3,791,932 and No. 3,839,153. In addition, a site-directed coupling reaction can be carried out so that any loss of activity due to polypeptide orientation after coupling can be minimized. See, for example, Rodwell et al., Biotech., 3:889-894 (1985), and U.S. Patent No. 4,671,958. Exemplary additional linking procedures include the use of Michael addition reaction products, di-aldehydes such as glutaraldehyde, Klipstein, et al., J. Infect. Dis., 147:318-326

(1983) and the like, or the use of carbodiimide technology as in the use of a water-soluble carbodiimide to form amide links to the carrier. Alternatively, the heterobifunctional cross-linker SPDP (N-succinimidyl-3-(2-pyridyldithio) propionate)) can be used to conjugate peptides, in which a carboxy-terminal cysteine has been introduced.

5 Useful carriers are well known in the art, and are generally proteins themselves. Exemplary of such carriers are keyhole limpet hemocyanin (KLH), edestin, thyroglobulin, albumins such as bovine serum albumin (BSA) or human serum albumin (HSA), red blood cells such as sheep erythrocytes (SRBC), tetanus toxoid, cholera toxoid as well as polyamino acids such as poly D-lysine:D-glutamic acid, and the like. The choice of carrier is more dependent upon the ultimate use of the inoculum and is based upon criteria not particularly involved in the present invention. For example, a carrier that does not generate an untoward reaction in the particular animal to be inoculated should be selected.

A suitable inoculum preferably comprises an effective (i.e., immunogenic) amount of a polypeptide or polypeptide fragment of the present invention, typically as a conjugate linked to a carrier. The effective amount of polypeptide per unit dose sufficient to induce an immune response to the immunizing polypeptide depends, among other things, on the species of animal inoculated, the body weight of the animal and the chosen inoculation regimen is well known in the art. Inocula typically contain polypeptide concentrations of about 10 micrograms (μg) to about 500 milligrams (mg) per inoculation (dose), preferably about 50 micrograms to about 50 milligrams per dose. The term "unit dose" as it pertains to the inocula refers to physically discrete units suitable as unitary dosages for animals, each unit containing a predetermined quantity of active material calculated to produce the desired immunogenic effect in association with the required diluent; i.e., carrier, or vehicle. The specifications for the novel unit dose of an inoculum of this invention are dictated by and are directly dependent on (a) the unique characteristics of the active material and the particular immunologic effect to be achieved, and (b) the limitations inherent in the art of compounding such active material for immunologic use in animals, as disclosed in detail herein, these being features of the present invention.

Inocula are typically prepared from the dried solid polypeptide-conjugate by dispersing the polypeptide-conjugate in a physiologically tolerable (acceptable) diluent such as water, saline or phosphate-buffered saline to form an aqueous composition. Inocula can also include an adjuvant as part of the diluent. Adjuvants such as complete Freund's adjuvant (CFA), incomplete

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Freund's adjuvant (IFA) and alum are materials well known in the art, and are available commercially from several sources.

The antibody so produced can be used, inter alia, in the diagnostic methods and systems of the present invention to detect a polypeptide of the present invention in a sample such as a tissue section or body fluid sample. Anti-polypeptide antibodies that inhibit function of the polypeptide can also be used in vivo in therapeutic methods as described herein. A preferred anti-polypeptide antibody is a monoclonal antibody. The phrase "monoclonal antibody" in its various grammatical forms refers to a population of antibody molecules that contain only one species of antibody combining site capable of immunoreacting with a particular epitope. A monoclonal antibody thus typically displays a single binding affinity for any epitope with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different epitope, e.g., a bispecific monoclonal antibody. A preferred monoclonal antibody of this invention comprises antibody molecules that immunoreact with a polypeptide of the present invention. More preferably, the monoclonal antibody also immunoreacts with recombinantly produced whole protein.

A monoclonal antibody is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) only one kind of antibody molecule. The hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. The preparation of such antibodies was first described by Kohler and Milstein, Nature, 256:495-497 (1975), the description of which is incorporated by reference. The hybridoma supernates so prepared can be screened for the presence of antibody molecules that immunoreact with a polypeptide.

Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with an antigen, such as is present in a polypeptide of this invention. The polypeptide-induced hybridoma technology is described by Niman et al., Proc. Natl. Acad. Sci., USA, 80:4949-4953 (1983), the description of which is incorporated herein by reference. It is preferred that the myeloma cell line used to prepare a hybridoma be from the same species as the lymphocytes. Typically, a mouse of the strain 129 GIX⁺ is the preferred mammal. Suitable mouse myelomas for use in the present invention include the

hypoxanthine-aminopterin-thymidine-sensitive (HAT) cell lines P3X63-Ag8.653, and Sp2/0-Ag14 that are available from the American Type Culture Collection, Rockville, MD, under the designations CRL 1580 and CRL 1581, respectively. Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 1500. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody of this invention are identified using the enzyme linked immunosorbent assay (ELISA) described in the Examples.

A monoclonal antibody of the present invention can also be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that produces and secretes antibody molecules of the appropriate polypeptide specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well known techniques. Media useful for the preparation of these compositions are both well known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's Minimal Essential Medium (DMEM; Dulbecco et al., Virology 8:396 (1959)) supplemented with 4.5 gm/l glucose, 20 mM glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c. Other methods of producing a monoclonal antibody, a hybridoma cell, or a hybridoma cell culture are also well known. See, for example, the method of isolating monoclonal antibodies from an immunological repertoire as described by Sastry, et al., Proc. Natl. Acad. Sci. USA, 86:5728-5732 (1989); and Huse et al., Science, 246:1275-1281 (1989).

The monoclonal antibodies of this invention can be used in the same manner as disclosed herein for antibodies of the present invention. For example, the monoclonal antibody can be used in the therapeutic, diagnostic or in vitro methods disclosed herein where immunoreaction with a nucleic acid, polypeptide or fragment thereof, as described herein, is desired. Also contemplated by this invention is the hybridoma cell, and cultures containing a hybridoma cell that produce a monoclonal antibody of this invention.

It is also possible to isolated antibodies reactive against polypeptides of the instant invention using phage display techniques. Display of antibody fragments on the surface of viruses which infect bacteria (bacteriophage or phage) makes it possible to produce human sFvs with a wide range of affinities and kinetic characteristics. To display antibody fragments on the

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surface of phage (phage display), an antibody fragment gene is inserted into the gene encoding a phage surface protein (pIII) and the antibody fragment-pIII fusion protein is expressed on the phage surface (McCafferty et al. (1990) Nature, 348: 552-554; Hoogenboom et al. (1991) Nucleic Acids Res., 19: 4133-4137). For example, a sFv gene coding for the V.sub.H and V.sub.L domains of an anti-lysozyme antibody (D1.3) was inserted into the phage gene III resulting in the production of phage with the D1.3 sFv joined to the N-terminus of pIII thereby producing a "fusion" phage capable of binding lysozyme (McCafferty et al (1990) Nature, 348: 552-554). The skilled artisan may also refer to Clackson et al. (1991) Nature, 352: 624-628), (Marks et al. (1992) Bio/Technology, 10: 779-783), Marks et al Bio/Technology, 10: 779-785 (1992) for further guidance. In the instant case, the antibody fragment gene is isolated from the immunized mammal, and inserted into the phage display system. Phage containing antibodies reactive to the polypeptide are then isolated and characterized using well-known techniques. Kits and services are available for generating antibodies by phage display from well-known sources such as Cambridge Antibody Technology Group plc (United Kingdom).

Autoantibodies to the polypeptides of the instant invention may also be detected using techniques well-known and widely available to the skilled artisan. For detection of autoantibodies in the serum of a patient by an antigen-antibody reaction, various conventional immunologically methods can be used such as a method of directly measuring a reaction in a liquid phase and a solid phase and a method of measuring an inhibitory reaction immunologically by adding an inhibiting substance. The following are the examples of the above-mentioned detecting methods, (1) aggregation reaction; (2) DID: double immune diffusion method (Ostergaard method); (3) ELISA: enzyme linked immunoabsorbent assay, (4) FIA: fluorescent immunosorbent assay, (5) nephelometry method, (6) radioimmuno assay (RIA), (7) immunofluorescent methods. Such methods are described in available references such as U.S. Pat. No. 5,976,810, incorporated herein by reference.

The presence of elevated levels of certain nucleic acids or polypeptides, such as *dek* in gliomas (see below) has potential for development of diagnostic reagents. *dek* has been shown to be an autoantigen in several diseases, such as juvenile rheumatoid arthritis, lupus erythematosus, and Kikuchi's Disease (Szer et al. *A novel autoantibody to the putative oncoprotein DEK in pauciarticular onset juvenile rheumatoid arthritis*. J Rheumatol 1994 Nov;21(11):2136-42; Wichmann et al.. *Autoantibodies to transcriptional regulation proteins*

DEK and ALY in a patient with systemic lupus erythematosus. Hum Immunol 1999 Jan;60(1):57-62; Sierakowska et al. The putative oncoprotein DEK, part of a chimera protein associated with acute myeloid leukaemia, is an autoantigen in juvenile rheumatoid arthritis. Clin Exp Immunol 1993 Dec;94(3):435-9; Murray et al. Antibodies to the 45 kDa DEK nuclear antigen in pauciarticular onset juvenile rheumatoid arthritis and iridocyclitis: selective association with MHC gene. J Rheumatol 1997 Mar;24(3):560-7; Dong et al. Autoantibodies to DEK oncoprotein in a patient with systemic lupus erythematosus and sarcoidosis. Arthritis Rheum 1998 Aug;41(8):1505-10; Arnaudo et al. Antibodies to the DEK protein in Kikuchi's disease. J Rheumatol 1998 Sep;25(9):1861-2). The present invention provides for the evaluation of the presence of *dek* autoantibodies in the serum of glioma patients. The existence of such autoantibodies may provide the foundation for both a novel non-invasive diagnostic for gliomas as well as a method for evaluation of tumor recurrence following treatment.

V. Methods of Treatment

15 a. Pharmacogenomics

The invention further provides for a method of ascertaining propensity for malignancy, monitoring the progress of chemotherapy or other anticancer therapy, screening for re-occurrence of cancer, or other similar detection of present or potential cancer, where such method detects for the expression of at least one gene which is over- or under-expressed in a cancer cell. In a preferred embodiment, the gene is nucleic acid sequence sharing substantial identity to a nucleic acid sequence selected from the sequences of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. The present invention provides for a method for ascertaining the propensity for malignant phenotype of cells in a biological sample, said method comprising assaying a biological sample to be tested for a signal indicating the transcription of a nucleic acid transcript, wherein said transcript is from at least one gene selected from the group consisting essentially of the genes encoded for by or containing the characteristic nucleic acid sequences identified in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

In a further embodiment of the invention, screening assays of biological samples are contemplated, where such assays are conducted during the course of chemotherapy alone, or after surgical intervention to treat cancer, to monitor for the continued presence or return of cancerous cells. Such screening assays are designed to detect for the presence of expressed nucleic acids

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corresponding to any of those listed in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof, as an indicator of the possible tumor recurrence. Such monitoring will quickly identify the effective anti-cancer drugs suitable for treatment of the identified brain cancer. In particular, such methods allow for identifying suitable combination therapies.

5 Related to the use described above, the methods and compositions of the present invention allow for a therapeutic prediction of the efficacy of any contemplated therapy or therapeutic on the specific brain cancer. By determining the characteristic gene expression features, and testing cells for modulation of such gene expression, it is possible to determine the potential responsiveness of the target brain cancer, to the proposed therapeutic.

10 Genetic screening is also made possible, as detecting mutations within the genes indicated by the nucleic acid sequences that are over- or underexpressed in a cancer cell. Preferably, the sequences are those in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. Using the sequences or the control elements of such genes, it is possible to detect and identify persons with a potential predisposition for cancer, and thus bring medical
15 monitoring early in the persons life.

 In another embodiment, the present invention provides for a method for monitoring the progression of cancer or the effectiveness of a treatment regimen in a patient. Changes in the expression of certain sequences indicates whether or not a treatment regimen is having an effect in the patient. For example, if a certain treatment regimen results in increased expression of a
20 sequence known to be associated with metastasis, it may be an indication that the treatment is not working to the benefit of the patient.

b. Gene Therapy

 The present invention further provides for methods of treating a patient by inhibiting or
25 introducing expression into the cells of a patient a nucleic acid or fragment thereof that shows increased or decreased expression in a tumor cell. The use of gene therapy to augment or ameliorate the expression of the genes associated with the nucleic acid sequences that are over- or under-expressed in tumor cells is also contemplated. In particular, the use of antisense molecules to interfere with mRNAs corresponding to the genes identified by such sequences. It
30 is also possible to construct recombinant DNA vectors which can affect targeted homologous recombination to delete or substitute such genes with normal or non-malignant forms. In a

preferred embodiment, the genes comprise sequence that is substantially identical to the sequences of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

In practicing the present invention, it is advantageous to transfect into a cell a nucleic acid construct directing expression of a protein or nucleic acid product having the ability to alter the behavior of the cell. There are available to one skilled in the art multiple viral and non-viral methods suitable for introduction of a nucleic acid molecule into a target cell. Genetic manipulation of primary tumor cells has been described previously (Patel et al., 1994. Human Gene Therapy 5, p. 577-584). Genetic modification of a cell may be accomplished using one or more techniques well known in the gene therapy field (Human Gene Therapy April 1994, Vol. 5, p. 543-563; Mulligan, R.C. 1993). Viral transduction methods may comprise the use of a recombinant DNA or an RNA virus comprising a nucleic acid sequence that drives or inhibits expression of a protein to infect a target cell. A suitable DNA virus for use in the present invention includes but is not limited to an adenovirus (Ad), adeno-associated virus (AAV), herpes virus, vaccinia virus or a polio virus. A suitable RNA virus for use in the present invention includes but is not limited to a retrovirus or Sindbis virus. It is to be understood by those skilled in the art that several such DNA and RNA viruses exist that may be suitable for use in the present invention.

Adenoviral vectors have proven especially useful for gene transfer into eukaryotic cells (Stratford-Perricaudet, L., and M. Perricaudet. 1991. *Gene transfer into animals: the promise of adenovirus*. p. 51-61, In: Human Gene Transfer, Eds, O. Cohen-Haguenauer and M. Boiron, Editions John Libbey Eurotext, France). Adenoviral vectors have been successfully utilized to study eukaryotic gene expression (Levrero, M., et al. 1991. *Defective and nondefective adenovirus vectors for expressing foreign genes in vitro and in vivo*. Gene 101: 195-202), vaccine development (Graham, F. L., and L. Prevec (1992) *Adenovirus-based expression vectors and recombinant vaccines*. In *Vaccines: New Approaches to Immunological Problems*, (Ellis, R. V. Ed.), pp. 363-390. Butterworth-heinemann, Boston), and in animal models (Stratford-Perricaudet, et al. 1992. *Widespread long-term gene transfer to mouse skeletal muscles and heart*. J. Clin. Invest. 90, 626-630; Rich, et al. 1993. *Development and analysis of recombinant adenoviruses for gene therapy of cystic fibrosis*. Human Gene Ther. 4, 461-476). The first trial of Ad-mediated gene therapy in human was the transfer of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to lung (Crystal, et al. 1994. Nature Genetics 8, 42-51).

- Experimental routes for administering recombinant Ad to different tissues *in vivo* have included intratracheal instillation (Rosenfeld, et al. 1992. *In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium*. Cell 68, 143-155) injection into muscle (Quantin, B., et al. 1992. *Adenovirus as an expression vector in muscle cells in vivo*. Proc. Natl. Acad. Sci. USA 89, 2581-2584), peripheral intravenous injection (Herz, J. and R.D. Gerard. 1993. *Adenovirus-mediated transfer of low density lipoprotein receptor gene acutely accelerates cholesterol clearance in normal mice*. Proc. Natl. Acad. Sci. USA 90, 2812-2816) and stereotactic inoculation to brain (Le Gal La Salle, et al. 1993. *An adenovirus vector for gene transfer into neurons and glia in the brain*. Science 259, 988-990). The adenoviral vector, then, is widely available to one skilled in the art and is suitable for use in the present invention.
- Adeno-associated virus (AAV) has recently been introduced as a gene transfer system with potential applications in gene therapy. Wild-type AAV demonstrates high-level infectivity, broad host range and specificity in integrating into the host cell genome (Hermonat, P.L., and N. Muzyczka. 1984. *Use of adeno-associated virus as a mammalian DNA cloning vector: transduction of neomycin resistance into mammalian tissue culture cells*. Proc. Natl. Acad. Sci. USA 81: 6466-6470). Herpes simplex virus type-1 (HSV-1) is attractive as a vector system for use in the nervous system because of its neurotropic property (Geller, A.I., and H.J. Federoff. 1991. *The use of HSV-1 vectors to introduce heterologous genes into neurons: implications for gene therapy*. In: Human Gene Transfer, Eds, O. Cohen-Haguenauer and M. Boiron, pp. 63-73, Editions John Libbey Eurotext, France; Glorioso, et al. 1995. *Herpes simplex virus as a gene-delivery vectors for the central nervous system*. In: Viral Vectors-Gene therapy and neuroscience application, Eds, M.G. Kaplitt and A.D. Loewy, pp. 1-23. Academic Press, New York). Vaccinia virus, of the poxvirus family, has also been developed as an expression vector (Smith, G.L., and B. Moss. 1983. *Infectious poxvirus vectors have capacity for at least 25,000 base pairs of foreign DNA*. Gene 25: 21-28; Moss, B. 1992. *Poxviruses as eukaryotic expression vectors*. Semin. Virol. 3: 277-283; Moss, B. 1992. *Poxviruses as eukaryotic expression vectors*. Semin. Virol. 3: 277-283). Each of the above-described vectors are widely available to one skilled in the art and would be suitable for use in the present invention.

- Retroviral vectors are capable of infecting a large percentage of the target cells and integrating into the cell genome (Miller, A.D., and G.J. Rosman. 1989. *Improved retroviral vectors for gene therapy and expression*. Biotechniques 7: 980-990). Retroviruses were

developed as gene transfer vectors relatively earlier than other viruses, and were first used successfully for gene marking and transducing the cDNA of adenosine deaminase (ADA) into human lymphocytes.

It is also possible to produce a viral vector in vivo by implantation of a "producer cell line" in proximity to the target cell population. As demonstrated by Oldfield, et al. (*Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir*, Human Gene Therapy 4:39-69), infiltration of a brain tumor with cells engineered to produce a viral vector carrying an effector gene results in the continuous release of the viral vector in the vicinity of the tumor cells for an extended period of time (i.e., several days). In such a system, the vector is retroviral vector which preferably infects proliferating cells, which, in the brain, would include mainly tumor cells. The present invention provides a methodology with which a viral vector supplies a nucleic acid sequence encoding a protein having sialyltransferase activity to cells involved in a neurological disorder such as brain cancer.

"Non-viral" delivery techniques that have been used or proposed for gene therapy include DNA-ligand complexes, adenovirus-ligand-DNA complexes, direct injection of DNA, CaPO_4 precipitation, gene gun techniques, electroporation, and lipofection (Mulligan, R.C. 1993. *The basic science of gene therapy*. Science 260: 926-932). Any of these methods are widely available to one skilled in the art and would be suitable for use in the present invention. Other suitable methods are available to one skilled in the art, and it is to be understood that the present invention may be accomplished using any of the available methods of transfection. Several such methodologies have been utilized by those skilled in the art with varying success (Mulligan, R.C. 1993. *The basic science of gene therapy*. Science 260: 926-932). Lipofection may be accomplished by encapsulating an isolated DNA molecule within a liposomal particle and contacting the liposomal particle with the cell membrane of the target cell. Liposomes are self-assembling, colloidal particles in which a lipid bilayer, composed of amphiphilic molecules such as phosphatidyl serine or phosphatidyl choline, encapsulates a portion of the surrounding media such that the lipid bilayer surrounds a hydrophilic interior. Unilamellar or multilamellar liposomes can be constructed such that the interior contains a desired chemical, drug, or, as in the instant invention, an isolated DNA molecule.

The cells may be transfected in vivo (preferably at the tumor site), ex vivo (following

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removal from a primary or metastatic tumor site), or in vitro. The cells may be transfected as primary cells isolated from a patient or a cell line derived from primary cells, and are not necessarily autologous to the patient to whom the cells are ultimately administered. Following ex vivo or in vitro transfection, the cells may be implanted into a host, preferably a patient having a neurological disorder and even more preferably a patient having a brain tumor. Genetic manipulation of primary tumor cells has been described previously (Patel et al., 1994. Human Gene Therapy 5, p. 577-584). Genetic modification of the cells may be accomplished using one or more techniques well known in the gene therapy field (Human Gene Therapy. April 1994. Vol. 5, p. 543-563; Mulligan, R.C. 1993. *The basic science of gene therapy*. Science 260: 926-932).

In order to obtain transcription of the nucleic acid of the present invention within a target cell, a transcriptional regulatory region capable of driving gene expression in the target cell is utilized. The transcriptional regulatory region may comprise a promoter, enhancer, silencer or repressor element and is functionally associated with a nucleic acid of the present invention. Preferably, the transcriptional regulatory region drives high level gene expression in the target cell. It is further preferred that the transcriptional regulatory region drives transcription in a cell involved in a neurological disorder such as brain cancer. Transcriptional regulatory regions suitable for use in the present invention include but are not limited to the human cytomegalovirus (CMV) immediate-early enhancer/promoter, the SV40 early enhancer/promoter, the JC polyomavirus promoter and the chicken β -actin promoter coupled to the CMV enhancer (Doll, et al. 1996. *Comparison of promoter strengths on gene delivery into mammalian brain cells using AAV vectors*. Gene Therapy 3: 437-447). Other transcriptional regulatory regions useful for practicing the present invention are available and well known in the art, and are contemplated as being part of the present invention.

The vectors of the present invention may be constructed using standard recombinant techniques widely available to one skilled in the art. Such techniques may be found in common molecular biology references such as *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), and *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA). Examples of nucleic acid constructs useful for practicing the present invention comprise

a transcriptional regulatory region such as the CMV immediate-early enhancer/promoter, the SV40 early enhancer/promoter, the JC polyomavirus promoter, or the chicken β -actin promoter coupled to the CMV enhancer operably linked to a nucleic acid comprising one or more of SEQ ID NOS. 1-184, or a fragment or complement thereof. To generate such a construct, a nucleic acid sequence encoding the enzyme may be processed using one or more restriction enzymes such that certain sequences flank the nucleic acid. Processing of the nucleic acid may include the addition of linker or adapter sequences. A nucleic acid sequence comprising a preferred transcriptional regulatory region may be similarly processed such that the sequence has flanking sequences compatible with the nucleic acid sequence encoding the enzyme. These nucleic acid sequences may then be joined into a single construct by processing of the fragments with an enzyme such as DNA ligase. The joined fragment, comprising a transcriptional regulatory region operably linked to a nucleic acid comprising a sequence that is over- or underexpressed in a cancer cell, preferably being a sequence substantially identical to a sequence of SEQ ID NOS. 1-184, or a fragment or complement thereof, may then be inserted into a plasmid capable of being replicated in a host cell by further processing using one or more restriction enzymes.

Administration of a nucleic acid of the present invention to a target cell *in vivo* may be accomplished using any of a variety of techniques well known to those skilled in the art. Such reagents may be administered by intravenous injection or using a technique such as stereotactic injection to administer the reagent into the target cell or the surrounding areas (Badie, et al. 1994. *Stereotactic Delivery of a Recombinant Adenovirus into a C6 Glioma Cell Line in a Rat Brain Tumor Model*. Neurosurgery 35: 910; Perez-Cruet, et al. 1994. *Adenovirus-Mediated Gene Therapy of Experimental Gliomas*. J. Neur. Res. 39: 506; Chen, et al. 1994. *Gene therapy for brain tumors: Regression of experimental gliomas by adenovirus-mediated gene transfer in vivo*. Proc. Natl. Acad. Sci. USA 91: 3054; Oldfield, et al. 1993. *Gene Therapy for Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir*. Human Gene Therapy 4:39-69; Okada, et al. 1996).

In another embodiment, the present invention provides a methodology for transfection of a functional nucleic acid sequence, preferably an antisense oligonucleotide, that inhibits expression of a nucleic acid comprising a sequence of SEQ ID NOS. 1-184, or a protein encoded by a nucleic acid comprising a sequence of SEQ ID NOS. 1-184. The antisense oligonucleotide may comprise a functional nucleotide sequence such as a 2',5'-oligoadenylate as described in

U.S. Patent No. 5,583,032. Using such an antisense oligonucleotide, expression of a protein comprising a sequence substantially identical to that encoded by the sequences of SEQ ID NOS. 1-184 may be inhibited by inhibition of transcription, destruction of the transcript encoding the protein, or inhibition of translation of the protein from its transcript.

5 In certain embodiments of the present invention, transfection of a cell is performed. In a preferred embodiment, the cell is involved in the causation of a neurological disorder such as brain cancer, Parkinson's disease or Alzheimer's disease. In a preferred embodiment, the cell is a cancer cell, and in a more preferred embodiment, the cell is a brain cancer cell. More preferably, the nucleic acid comprises a sequence encoding the protein encoded by a nucleic acid
10 comprising a sequence of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof is under the transcriptional control of a transcriptional regulatory region which functions within a neural tissue or cell.

In another embodiment of the present invention, a target cell is transfected in vivo by implantation of a "producer cell line" in proximity to the target cell population (Oldfield, et al.
15 1993. *Gene Therapy for Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir*. Human Gene Therapy 4:39-69; Culver, et al. 1994. *Gene Therapy for the Treatment of Malignant Brain Tumors with in vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System*, Human Gene Therapy 5: 343-379). The producer cell line is engineered to produce a viral vector and releases
20 viral particles in the vicinity of the target cell. A portion of the released viral particles contact the target cells and infect those cells, thus delivering a nucleic acid of the present invention to the target cell. Following infection of the target cell, expression of the product of nucleic acid of the present invention occurs. Preferably, expression results in either increased or decreased expression of a protein encoded by the nucleic acid, which preferably comprises substantially
25 identical DNA sequence to the sequences of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

In yet another embodiment, the present invention comprises a kit for determining the tumorigenicity or malignancy of a brain cell. The kit may comprise a panel of independent or
30 paired nucleic acid molecules specific for the detection of the expression of specific nucleic acid sequences corresponding to nucleic acid sequences that are over- or under-expressed in cancer cells. Preferably, the sequences are substantially identical to those of SEQ ID NOS. 1-184,

sequences complementary thereto, or fragments thereof. One embodiment of such a kit utilizes enzyme-mediated nucleic acid amplification such as the polymerase chain reaction (PCR) in which a pair of nucleic acid molecules (i.e., primers) that allow for amplification of a nucleic acid sequence of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof.

5

c. Small Molecules

The methods and compositions of the present invention are useful for the manufacture of pharmaceuticals and therapeutics which encompass compounds that interact with or affect the expression of nucleic acid sequences or proteins over- or underexpressed in cancer cells. Preferably, the nucleic acid sequences comprise sequence substantially identical to those sequences listed in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. Such inhibitors can take the form of traditional chemotherapeutic agents, as well as specific anti-sense nucleic acids targeted to the nuclei acid sequences. Such therapeutics can be directed against single nucleic acid targets, but most preferably are targeted at more than one specific nucleic acid sequence.

The present invention also provides for therapeutic compounds identified or otherwise identifiable by this method, and any compound corresponding to a compound identified by these methods. The reagents and methodologies of the present invention provide an assay system for determining the effect of a compound on gene expression in a cell. In one embodiment, the cell may be affected such that upon administration of the compound to a patient, cell growth or activity that may be detrimental to the patient may result. In such cases, it would be beneficial to have at the researcher's disposal a rapid, accurate, and efficient assay system to measure the likelihood that a compound may have such effects. Preferably, the "panel" refers to the sequences substantially identical to one or more of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. It is to be understood by the skilled artisan that the present invention provides an assay or test system that is applicable to many types of cells and panels of nucleotide sequences.

In one embodiment, the present invention provides an assay for identifying a compound that may promote or prevent cancer. A method for identifying a compound affecting a cell is provided wherein a cell is contacted with a compound and expression of one or more nucleotide sequences or proteins selected from a panel of sequences is detected. The panel may consist of

one or more sequences of the invention. The level of expression may be compared to control levels, such as where a cell has not been contacted by the compound but is otherwise maintained under identical conditions as the cell that has been contacted. In one embodiment, a method for detecting a compound that may promote cancer comprising detection of increased expression of the panel of sequences following contact of the cell with the compound is provided. In another embodiment, a method for detecting decreased expression of one or more members of the panel of sequences following exposure to the compound, thus identifying a compound that may inhibit tumor cell migration. In yet another embodiment, a method for detecting increased expression of the one or more members of the panel following exposure to the compound, thus identifying a compound that may promote tumor cell migration. In a preferred embodiment, the present invention provides an assay for identifying a compound that may promote or prevent brain cancer. In one embodiment, the sequences are selected from sequences substantially identical to those sequences in SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. Any combination of such sequences may be combined to provide a useful assay system as described herein.

In one embodiment of the present invention, a method for identifying a compound affecting a cell is provided wherein a cell is contacted with a compound and expression of a reporter gene functionally linked to a transcriptional regulatory sequence of a nucleotide sequence that is up- or down-regulated in cancer cells. In a preferred embodiment, the reporter sequences is β -galactosidase, luciferase, green fluorescent protein or chloramphenicol acetyl transferase (CAT). In a preferred embodiment, the transcriptional regulatory region controls the expression of a sequence substantially identical to a sequence of SEQ ID NOS. 1-184, sequences complementary thereto, or fragment thereof.

In yet another embodiment, the present invention comprises a kit for determining the effect of a compound on gene expression within a cell. The kit may comprise packaged reagents such as a panel of independent or paired nucleic acid molecules specific for the detection of the expression of specific nucleic acid sequences corresponding to specific species of nucleic acid sequences encoding proteins of interest. Instructions for use of the packaged reagent(s) are also typically included. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene or polycarbonate), paper, foil and the like, capable of holding within fixed limits a polyamide of the present invention. "Instructions for use"

typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent or sample admixtures, temperature, buffer conditions and the like.

5 In another embodiment, the present invention provides a compound identified by its ability to cause an increase or a decrease in one or more sequences of a panel of sequences. The compounds of this invention may be formulated into diagnostic and therapeutic compositions for *in vivo* or *in vitro* use. Representative methods of formulation may be found in *Remington: The Science and Practice of Pharmacy*, 19th ed., Mack Publishing Co., Easton, PA (1995). For *in vivo* use, the compound may be incorporated into a physiologically acceptable pharmaceutical composition that is administered to a patient in need of treatment or an animal for medical or research purposes. The polyamide composition comprises pharmaceutically acceptable carriers, excipients, adjuvants, stabilizers, and vehicles. The composition may be in solid, liquid, gel, or aerosol form. The polyamide composition of the present invention may be administered in 10 various dosage forms orally, parentally, by inhalation spray, rectally, or topically. The term parenteral as used herein includes, subcutaneous, intravenous, intramuscular, intrasternal, infusion techniques or intraperitoneally.

The selection of the precise concentration, composition, and delivery regimen is influenced by, *inter alia*, the specific pharmacological properties of the particular selected 20 compound, the intended use, the nature and severity of the condition being treated or diagnosed, the age, weight, gender, physical condition and mental acuity of the intended recipient as well as the route of administration. Such considerations are within the purview of the skilled artisan. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods.

25 The pharmaceutically active compounds (i.e., polypeptides, nucleic acids, compounds or vectors) of this invention can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals. For oral administration, the pharmaceutical composition may be in the form of, for example, a capsule, a tablet, a suspension, or liquid. The pharmaceutical composition is 30 preferably made in the form of a dosage unit containing a given amount of DNA or viral vector particles (collectively referred to as "vector"). For example, these may contain an amount of

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vector from about 10^3 - 10^{15} viral particles, preferably from about 10^6 - 10^{12} viral particles. A suitable daily dose for a human or other mammal may vary widely depending on the condition of the patient and other factors, but, once again, can be determined using routine methods. The vector may also be administered by injection as a composition with suitable carriers including saline, dextrose, or water.

Injectable preparations, such as sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

A suitable topical dose of active ingredient of a vector of the present invention is administered one to four, preferably two or three times daily. For topical administration, the vector may comprise from 0.001% to 10% w/w, *e.g.*, from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w, but preferably not more than 5% w/w, and more preferably from 0.1% to 1% of the formulation. Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin (*e.g.*, liniments, lotions, ointments, creams, or pastes) and drops suitable for administration to the eye, ear, or nose.

The pharmaceutical compositions may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions, or emulsions). The pharmaceutical compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc. Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills,

the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings. Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise
5 adjuvants, such as wetting sweetening, flavoring, and perfuming agents.

The compositions of the present invention may be administered orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intramuscular, intrasternal, infusion techniques or
10 intraperitoneally. Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable non-irritating excipient such as cocoa butter and polyethylene glycols that are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

The dosage regimen for compositions of this invention is based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity
15 of the condition, the route of administration, and the particular compound employed. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods.

While the compounds, polypeptides, nucleic acids and /or vectors of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination
20 with one or more vectors of the invention or other agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

VI. Conclusions

25 Thus the compositions and methods of the present invention are useful as clinical screens for the specific diagnosis and identification of cancer. Preferably, the cancer is brain cancer, and more preferably, the cancer is glioma. In one embodiment, the strong indication of glioma is characterized by detection of increased or decreased expression of SEQ ID NOS. 1-184, sequences complementary thereto, or fragments thereof. The methods and assays of the
30 invention are also useful for the detection of potential cancer development such as glioma or other cancers. Thus the determination and early detection of glioma propensity greatly assists

the medical practitioner and patient decide upon the proper course of action. Once such action is taken, the methods of the present invention allows for the monitoring of recurrence after surgery, or during the course of chemotherapy.

5 The following Examples are for illustrative purposes only and are not intended, nor should they be construed as limiting the invention in any manner. Those skilled in the art will appreciate that variations and modifications can be made without violating the spirit or scope of the invention.

EXAMPLES

10 As discussed above, DDRT-PCR is a powerful method for identifying and analyzing altered gene expression at the mRNA level. It has been utilized to identify cellular mRNAs whose expression is altered in malignant brain tumors, and has successfully yielded several genes. Most of these to date are still of unknown function and clinical utility. Established herein is a reliable DDRT-PCR/screening protocol to study modulation of gene expression in human
15 brain tumors. A comparison between cultured NHFA and a tumorigenic glioma cell line, U373MG was initially chosen for study. This system provided a proliferative model of glial lineage which supplied both well-defined and renewable resources necessary for our intensive screening protocols. Following DDRT-PCR using a panel of 84 unique primer pairs, differentially expressed amplicons were further screened by a series of Northern analyses. As
20 described below, comparison of cultured normal human fetal astrocytes (NHFA) with a tumorigenic glioma cell line (U373MG) initially generated at least 142 differentially expressed transcripts, wherein at least SEQ ID NOS. 1-94 appeared to be under-expressed in the tumor cells. In addition, at least SEQ ID NOS. 95-141 and 183 appear to be over-expressed in tumor cells. SEQ ID NO. 68, 69 and 183 were further confirmed by reverse northern blot.

25 Age at primary diagnosis is among the most significant factors impacting survival of patients with glioblastomas (GBM). Patients diagnosed prior to the age of 50 years survive significantly longer than those diagnosed after the age of 50, with median survival of 24 months and 8 months, respectively. This differential survival is independent of performance status and appears to be unrelated to treatment. The cellular mechanisms for this age/prognosis correlation
30 are not known. Several age-related genetic alterations have been recently demonstrated in malignant gliomas, suggesting that there is a molecular basis for this poor patient survival.

Overall survival of patients diagnosed with GBMs demonstrates a marked inverse age-dependence (**Figure 1**). In order to understand the molecular basis for this poor patient survival, we utilized a DDRT-PCR-based strategy and identified multiple differentially expressed mRNAs in GBMs excised from older (>60 yr.) and younger (<45 yr.) patients. As shown below, DDRT-PCR indicates that SEQ ID NOS. 142-174 are over-expressed in tumors from old patients as compared to those of young patients. SEQ-ID NOS. 175-182 were determined to be under-expressed in tumors of old patients as compared to those of young patients. The expression of SEQ ID NOS. 142, 143, 144, 147, 149, 162 and 173 were confirmed by reverse northern blot.

Example 1

Isolation of RNA

Human glioblastoma cell line U373MG (American Type Culture Collection - ATCC, Manassas, VA) was the source of malignant phenotype expression signals. Cultured normal human fetal astrocytes, isolated according to Yamamoto et al., (1997, Brain Research 755(1):175-9), and processed no later than 20 passages from the initial isolation, was the source of normal tissue expression signals. All cells were subcultured in Dulbeccos Modified Eagles Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS; Whittaker BioProducts, Walkersville, MD), penicillin/streptomycin and glutamine and were maintained in log phase at 37°C in the presence of 10% CO₂.

The material for the secondary clinical reverse northern screens was obtained with informed consent from two sources: (1) normal human brain tissue was obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland, (Baltimore, MD), (2) human brain tumor tissue, from donor tissue, glioblastoma multiforme, recurrent glioblastoma multiforme, and astrocytoma grade IV (glioblastoma) was obtained from excised tumor material. The clinical material, as classified according to WHO Brain Tumor Classification, are all treated as glioblastoma tissue.

Briefly, total RNA was extracted from tissues by guanidinium thiocyanate treatment, followed by separation using cesium chloride centrifugal sedimentation, and treated with DNase I for 30 minutes at 37°C.

RT-PCR was performed on the extracted RNA using commercially available oligonucleotide primers, following the recommended procedures. Specifically, anchored primers

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and 20 arbitrary 10-mer primers from Operon Technologies, Inc. (Kit A; Alameda, CA), and 8 arbitrary 13-mer primers from GenHunter Corp. (Cat. No. H-AP-D; Brookline, MA) were selected. Specifically, the primers were:

- 5 Anchored Primer $T_{11}M$ (where M is A, C or G)
 Random Primer Operon Technologies, Kit A, primers OPA-01 to OPA-20
 GenHunter Corp., H-AP primer set 4, primers H-AP25 to H-AP32

10 The combination of the primers from these two commercial kits produce a total of 84 unique primer pairs. Differential display was performed essentially as described by Liang et al., (*Science*, 1992, 257:967-71). For each of the three anchored primers in each sample, 0.2 ug of total DNA-free RNA was reverse transcribed with 50U Maloney Murine Lukemia Virus (MMLV) reverse transcriptase in the presence of 200 pmol anchored primer, and 20 uM dNTP for 5 minutes at 65°C, followed by 60 minutes at 37°C. Following heat inactivation of the reverse transcriptase at 75°C for 5 minutes, 2 µl of the RT mixture was amplified in the presence of 2
 15 α- ^{32}P dATP (1000-3000 Ci/mmol; Amersham Corp., Arlington Hts., IL), and 1 Unit of AmpliTaq^(R) (*T. aquit.* DNA polymerase; Perkin-Elmer Corp., Branchburg, NJ). The cycling parameters were: 94°C for 15 sec., 40°C for 2 min., 72°C for 30 sec., for 40 cycles. Following a final extension for 5 min. at 72°C, the samples were stored at 4°C until analysis. The PCR
 20 products were electrophoresed on 6% sequencing gels. Differentially expressed bands of interest were excised from the dried gel, boiled in dH₂O, purified by ethanol precipitation, and reconstituted in 10 µl dH₂O.

 The minimal selection criteria for the bands of interest was approximately two-fold greater signal expressed in either tissue, and was qualitatively evaluated by visual inspection of the autoradiographic image.

25

Example 2

Characterization of Sequences

 An aliquot of the purified cDNA amplicons were then reamplified and subcloned into the cloning site of a cloning vector and insert-containing vectors from multiple positive
 30 transformants were sequenced using an ABI 377 automated fluorescence-based nucleic acid sequencer. All NCBI maintained nucleotide databases (National Center for Biotechnology

Information; Bethesda, MD) were searched for homologies using the BLAST (basic local alignment search tool) program. The following sequences were identified as being under-expressed in tumor tissue as compared to normal tissue (SEQ ID NOS. 1-94) or over-expressed in tumor tissues as compared to normal tissues (SEQ ID NOS. 95-141).

- 5
 SEQ ID NO. 1: NA1-1-N
 TCAGGCCCTTCATGTTAGTAAAAGCAGACAGACTTTTATATAAAGCCCAGCTTTACCTTTTACTTATTAGTTTGA
 ATGAACTTGGGCAAGTTACTTAGTTTTCTGAATCTCATTTTTTCAAATGAAAATTAATTCATATAATTCCTTCT
 10 CTAGGGGATTTAATTATTATTAGAGACAGGGTCTCAGTGTGTACATAGGCTGGAGTGCAGTGGTGTGATCATAG
 CTCATCGTATCCTCAAACACTTGGCTCAAGCAATCCCCCTGCCTCAGCCTCCTAAGTAGCTAGGACTACAGGCC
 TGTGCCACCTTGCTGGCTAACTAAAAAAAAGCTT
- 15
 SEQ ID NO. 2: NA1-1-P
 CAGGCCCTTCCAAAAAATAGAAGTGGAGGAAACAATTCTTAACACATTCCTTGAGGCCAGCATTACCGTGGTAG
 CTGAGCCCGATAAAAAATGGTCATAGAAGAGAAAAATCACAAACCATATCCCTTATCAATGTAGATGCTAAAAATTTT
 CCACAGAATACCAGCAAACTTAATCCAACAGTGTATTAAGGTTTAGACTTGTCTCAGGTGGGATTTATTTCCA
 GGAATGTAAAGTGGTTCAGTTTAAAGAAATTAATTAACACTACCTGCACATCTCAGTTGACACACGAAAGGTGT
 CTGACAAATCTCATAACTGTTTCATGATAAAAAAAAAGCTT
- 20
 SEQ ID NO. 3: NA2-1-F,G,H
 TTGCCGAGCTGGAATTGGAAGAAGGTGATGACGCAATCTGCCTCGCAGAGTTGAAGTTGGGCTTCATAGCCCAG
 AGCTGCCTGGCTCAAGGCCCTCTCCACCATGCTTGCCAACCTCTTCTCCATGAGGTCAATCATAAAGATTGAGGAA
 GACACATGGCAGAAATACTACTTGGAGGAGTCTCAAATGAAATGTACACAGAATATCTCTCCAGTGCCTTCGTG
 25 GGTCTGCTCTCCCTACTGTTTGTGAGCTGTGTTTTGTGAAGCTCAAGCTCCTAATGATAGCCATTGAGTACAAG
 TCTGCCAACCGAGAGAGCCGAAGCCGAAAGCGTATATTAATTAATCCTGGAACCATCTTAAGATCCAAGAAGGT
 ACTTAGGATTTTTTCATCGCAAGTGATGCCAAAGAAGTTAAAGGGCATCTTTTACTGCAAGGCTGTCTGAT
 GACATCACAGATCCCAAAGAATAAAAAATGTGGCTGCAACGGCTTGAAGATGAGCAGCCCGTCAACACTATC
 ACCAAAAAAAAGCTT
- 30
 SEQ ID NO. 4: NA5-1-F,H
 AGGGGTCTTGCAGAAATGAATTAACCTGAATTCAACAAAAGAGGTCTTTAAATTCATAACAGCAGGTGTCGTCT
 GTCTTTGAGATTCCCTTGCCAAAAAAGGAAATGATTTCTTAGTGATATGCTTTACTTCTGTTGATCACTATTTGC
 TCTTTTAAAGTGCCAAAGATGTTTTAATAGATACTTGGTATTTGTTGTTTTCTTTAATAAAGTATAATTTACAT
 35 GTAAAAAAAAGCTT
- SEQ ID NO. 5: NA5-1-G
 AGGGGTCTTGGCACAGGAAAAGGACAGTAGGTCAAACCTAAGGAATATCAATGAAGTATGGGCCTTAGTTAATAT
 TAAAGTATCAATATTGGTATATTAGTTGTATCAAATGTATCATACTAATGTAAGATATTAACCATAGGGGAACT
 40 GCCTGTGACATACATGGAATTTCTGTACAAATTTCTGTAAATCTAAATATTTTAGAATAGAAGGCTATTT
 AAAAAAAAAGCTT
- SEQ ID NO. 6: NA10-1-A,B
 GACCGCTTGTGAATGCAACAAAATTCAAATTTCCCTGAAAATTTATTCAACTTCTATATGCCAAGCACACCGCT
 45 AAAGGCTTATCTTCTAAGTATATGCAGGCATACCTACTCACAAATAGCTTATTACCAGAGATAGGAAATTCG
 AGGTAATTTGGGAGAAATGTCATAGCCAAATTTATGGAAAAATAAAATAAAACTTCTCTATGGCCTCTTGAT
 TTAAGAAAAAACAGAACATACTAAAAAAAAGCTT
- 50
 SEQ ID NO. 7: NA11-4-A
 CAATCGCCGTTAGAATATACGTGACCACTGGTATTAGCTACTTCTGCCAATAGGGGGCATTGTTTTGAGAAAA
 ACAGCAGTCAGATTTCGTCCAGATGTCTACCTAAGGGTTCCTGGCAAAGGGGAGTCATTGTCCGAGACCTCAGTT
 GCTTGCCTTTTGGAAATTTGATGGCCTCTAGGTGTGAGAAAGAAAAAACTTCCATAAGGTTAGATACGCAGGG
 GTAAACATGTATTATAGTAAAGAAATTTAGTGCCAAAGATTTAGAAATAAAAGTGAATATACTAATTAT
 TCTAAAAAAAAGCTTAAGGCGAATTC
- 55
 SEQ ID NO. 8: NA11-4-B,C
 GCCCTTAAGCTTTTTTTTTTACATCACTTTAGAAATTTTATTGTATTCCTTAATGCATTTCTTAACATGTATAG
 CACTCTTTAATCAAGAAATATAAGTCATCTACTAGAAATCACATTATCTTAAAGATGCATAGTGAATGATAAGT
 TTGAAGATGTAATCAACAATTTCTTCAAATCATATCAATATATTACTCTCATGGAAGTGCACATTCTAA

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GAAGGGTCATTTTTTCCCCCAGTACTGGGAAGGTATGCATTTAACCATGTGGTCAGCCAGAAAGGCTGTTTTAT
ATATGGTGTGTGTACGGCGATTG

5 SEQ ID NO. 9: NA12-1-A,B,C
AAGCTTTTTTTTTTACACTGGAAGGGTCCGATTGCTGGTAAATATGGCTCTATCTATCGCCGACTATCGCCCCAC
TATCACCCACTATCGCCGAAGGGCGAATTC

10 SEQ ID NO. 10: NA15-1-A,L
TTCCGAACCCACTCCACCTTACTACCAGACAACCTTAACCAAACCATTTACCCAAATAAAGTATAGGCGATAGAA
ATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAGATGAAAAATTATAACCAAGCATAATATAGCAAGG
ACTAACCCTATACCTTCTGCATAATGAATTAAGTAAATAACTTTGCAAGGAGAGGCCAAAGCTAAGACCCCCG
AAACCAGACGAGCTACCTAAGAAGAGCTAAAAGAGCACACCCGCTCTATGTAGCAAAATAGTGGGAAGATTATAG
GTAGAGGCGACAAACCTACCGAGCCTGGTGATAGCTGGTTGTCCAAGATAGAATCTTAGTTCAACTTTAAATTG
CCCACAGAACCCTCTAAATCCCCTTGTAATTTAACTGTAAAAAAAAGCTT

15 SEQ ID NO. 11: NA15-1-H
TTCCGAACCCATACTTTAGAGTACATACCAGTAAATTCCAAATAAATTTAAATATAAAACATAAACC
ATATATGTAATGTGAATTAATTTTATATGCTTGGGGTAGTAAAGGGCTTTTATAATATGGTTTGAATCCAG
ATGCCATGAAAGAGAAAATTAATACATTTTCTACACAAGAGTAAACATTTCTGCATGGCAAAACGTGAAAGTAA
AGTCAAAACATAAATAACAAAGAGGTAAAAACTTTTGTGCTTCATATCCAGATAGTAAATAATTTTCTAATGT
20 AAAAGAACTCAAATTACTTCATAGAAGACTAAATATCAACAAAAATAGAGTAAAGATATCAACAGAGGGTT
CAAGGAAAAGTAAGTACAAAACTTATACAATATTTGAAGTGCTCAAACCTATTATTAATAAAAAAAGCTT

25 SEQ ID NO. 12: NA16-3-Q
ANCCAGNGAAACCACAGCCAAGGGAACGGGCTTGGCGGAATCAGCGGGGAAAGAAGACCTATTNTTCATANCCN
ANTACNCAACATTATTANAATAAACACCCCTCACCCTACAATCTTCTTANGAACAAACATATGACGCACTCTCCC
CTNAACTCTACACAACATATTTNGTCACCAAGACCCCTACTTCAACCTCCCTGNTCTTAT

30 SEQ ID NO. 13: NA16-3-M
AAGCTTTTTTTTTTAAAGAGGAAAACCCGGTAATGATGTGCGGGTTGAGGGATAGGAGGAGAATGGGGGATAGGT
GTATGAACATGAGGGTGTCTCGTGTGAATGAGGGTTTTATGTTGTTAATGTGGTGGGTGAGTGAGCCCCATT
GTGTTGTGGTAAATATGTAGAGGGAGTATAGGGCTGTGACTAGTATGTTGAGTCTGTAAGTAGGAGAGTGATAT
TTGATCAGGAGAACGTGGTTACTAGCACAGAGAGTCTCCAGTAGGTTAATAGTGGGGGTAAGGCGAGGTTAG
CGAGGCTTGCTAGAAGTCATCAAAAAGCTATTAGTGGGAGTAGAGTTTGAAGTCCTTGAGAGAGGATTATGATGC
35 GACTGTGAGTGCCGTTGCTAGTTTGTAGTCAAGCTCAACAGGGTCTTCTTTCCCGCTGATTCCGCCAAGCCCGTT
CCCTTGGCTGTGGTTTCGCTTGGCTAANGGCGAATTCAGCACACTGGCGGCCCTACTANTGGATCCCCAAGCTC
GGTACCAAGCTTTGATGCATAGCTTGAGTATTCTATAGNGNCCCTAATANCTTGGCCTAATCATGGCCATANCT
GGTTCTGNGNGAAATTGGTATNCGNTCACAATTNCCCAACAACNTCCGAA

40 SEQ ID NO. 14: NA16-3-P
GCCAGCGAAACCACAGCCAAGGGAACGGGCTTGGCGGAATCAGCGGGGAAAGAAGACCTGTTGAGCCTGAACCT
TACNCAACATATTTGNCAACCAAGACCCCTACTTCAACCTCCCTGTTCTTAT

45 SEQ ID NO. 15: NA16-3-L
GCCAGCGAAACCACAGCCAAGGGAACGGGCTTGGCGGAATCAGCGGGGAAAGAAGACCTGAACCTCTACACAACA
TATTTTGTACCAAGACCCCTACTTCAACCTCCCTGTTCTTATGAATTCGAACAGCATACCCCGATTCCGCTAC
GACCAACTCATAACCTCCTATGAAAAAAGCTTCCCTACCCTCACCCTAGCATTAATATGATATGTCTCCATA
CCCATTAATCTCCAGCATTTCCCTCAAACTAAAAAAAAGCTT

50 SEQ ID NO. 16: NA16-3-O
AGCCAGCGAAACCACAGCCAAGGGAACGGGCTTGGCGGAATCAGCGGGGAAAGAAGACCAACCGAACCCCTTCG
ACCTTGCCGAAGGGGAGTCCGAACCTAGTCTCAGGCTTCAACATCGAATACGCCGAGGCCCTTCGCCCTATTCT
TCATAGCCGAATACACAAACATTATATAATAAACACCCCTCACCCTACAATCTTCTAGGAACAACATATGACG
CACTCTCCCTGAACTCTACACAACATATTTGTACCAAGACCCCTACTTCAACCTCCCTGTTCTTATGAATTC
55 GAACAGCATACCCCGATTCCGCTACGACCAACTCATACACCTCCTATGAAAAAAGCTTCCCTACCCTCACCCTAG
CATTACTTATATGATATGTCTCCATACCCATTACAATCTCCAGCATTTCCCTCAAACTAAAAAAAAGCTT

60 SEQ ID NO. 17: NA16-3-K
AAGCTTTTTTTTTTAAATAGAAATGTGAAGATGATAAGTGTAGAGGGAAGGTTAATGGTTGATATTGCTAGGGT
GGCGCTTCCAATTAGGTGCATGAGTAGGTGGCTGCAGTAATGTTAGCGGTTAGGCGTACGGCCAGGGCTATTGG
TTGAATGAGTAGGCTGATGGTTTCGATAATAACTAGTATGGGATAAGGGGTGAGGTGTGCTTGTGGTAAGAA

GTGGGCTGGGGCATTTTTAATCTTAGAGTCAAGCTCAACAGGGTCTTCTTTCCCCGCTGATTCCGCCAAGCCCGT
TCCCTTGGGTGTGGTTTCGCTGGCT

5 SEQ ID NO. 18: NA16-3-I
AAGCTTTTTTTTTTTAAGAGGAAAACCCGGTAATGATGTCGGGGTTGAGGGATAGGAGGAGAATGGGGGATAGGT
GTATGAACATGAGGGTGTCTCGTGTGAATGAGGGTTTATGTTGTTAATGTGGTGGGTGAGTCAAGCTCAAC
AGGGTCTTCTTTCCCCGCTGATTCCGCCAAGCCCGTTCCTTGGCTGTGGTTTCGCTGGCT

10 SEQ ID NO. 19: NA16-3-N
AGCCAGCGAAACCACAGCCAAGGGAACGGGCTTGGCGGAATCAGCGGGGAAAGAAGACCCCTATTCTTCATAGCCG
AATACACAAACATTATTAGAATAAACACCCCTCACCCTACAATCTTCCCTAGGAACAACATATGACGCACTCTCCC
CTGAACCTACACAAACATATTTGTCCCAAGACCCCTACTTCAACCTCCCTGTTCTTATGAATTGGAACAGCAT
ACCCCGGATTCCGCTACGACCACTCATACACCTCCTATGAAAAAATCTCTACCACTCACCTAGCATTACTTA
15 TATGATATGTCTCCATACCCATTACAATCTCCAGCATTCCCCCTCAAACCTAAAAAAGCTT

20 SEQ ID NO. 20: NA16-3-R
AAGCTTTTTTTTTTTAAGAGGAAAACCCGGTAATGATGTCGGGGTTGAGGGATAGGAGGAGAATGGGGGATAGGT
GTATGAACATGAGGGTGTCTCGTGTGAATGAGGGTTTATGTTGTTAATGTGGTGGGTGAGTGAAGCCCAT
GTGTGTGGTAAATATGTAGAGGGAGTATAGGGCTGTGACTAGTATGTTGAGTCTGTAAAGTAGGAGAGTGATAT
TTGATCAGGAGAACGTGGTTACTAGCACAGAGATTCTCCAGTAGGTTAATAGTGGGGGTAAAGCGAGGTTAG
CGAGGCTTGCTAGAAGTCATCAAAAAGCTATTAGTGGGAGTAGAGTTTGAAGTCTTGAGAGAGGATTATGATGC
GACTGTGAGTGCGTTCGTAGTTTGTAGTTGCTAGACTAGGTAAGCTCAACAGGGTCTTCTTTCCCCGCTGATT
CCGCCAAGCCCGTTCCTTGGCTGTGGTTTCTGGCT

25 SEQ ID NO. 21: NA16-4-A
GACCGCTTGACTGAAGGGAACAGAGACAGAATGAAATGAAAGAAGGCAGTTGAACTTCTAGGCTTCTACAGGCA
GAAACAGGCTGATAGAAGTGTCACTACAGACATGTTCTACCTTTCTAGAAAAAAGCTTAAGGGCGAA
TTC

30 SEQ ID NO. 22: NA16-4-Q
AANCTTTTGTNTTTATGNGTTGGCNGCAGGTNGAGGCTTACTAAAAGNGTGAAAACGTATGCTTGGATTAAAGG
CTGACAGCGATTGCTAANGATAGTCAGTANAATTANAATTGTGAAGATGATAANTGTAGAGGGAAGGTTAATGGT
TGATATTGNTAGGGTGGCNCNTNCNNNTTAGNTGCCNNACTANANTNAAGCTNAACAGGGTCTTCTTTCCCNNTG
35 NTTCCGCAAGCCCGTNCCTTGGCTGNGGTTNCNCTGGCT

SEQ ID NO. 23: NA16-4-N
AAGCTTTTTTTTTTTATAAGATTATTAGTATAAAAGGGGAGATAGGTAGGAGTAGCGTGGTAAGGGCGATGAGTG
TGGGGAGGAATGGGGTGGGTTTGTATGTTCAAACCTGTCATTTTATTTTACGTTGTTAGATATGGGGAGTAGTG
40 TGATTGAGGTGGAGTAGATTAGGCGTAGGTAGAAGTAGAGGTTAAGCTCAACAGGGTCTTCTTTCCCCGCTGATT
CCGCCAAGCCCGTTCCTTGGCTGTGGTTTCGCTGGCT

SEQ ID NO. 24: NA16-4-K
AAGCTTTTTTTTTTTAAGAGGAAAACCCGGTAATGATGTCGGGGTTGAGGGATAGGAGGAGAATGGGGGATAGGT
GTATGAACATGAGGGTGTCTCGTGTGAATGAGGGTTTATGTTGTTAATGTGGTGGGTGAGTGAGCCCAT
45 GTGTTGTGGTAAATATGTANAGGGAGTATAGGGCTGTGACTAGTATGTTGAGTCTGTAAAGTAGGAGAGTGATAT
TTGATCAGGAGAACGTGGTTACTAGCACAGAGAGTTCTCCAGTAGGTTAATAGTGGGGGTAAAGCGAGGTTAG
CGAGGCTTGCTANAAGTCATCAAAAAGCTATTAGTGGGAGTAGAGTCAAGCTCAACAGGGTCTTCTTTCCCCGCT
GATTCCGCAAGCCCGTTCCTTGGCTGTGGTTTCNCTGGCT

50 SEQ ID NO. 25: NA16-4-1
ANNCNTNGNNNNNNNANCAANGGGAACGGGCTTGGNGGAATCAGCNGNGAANAAGACCCCTNANNTCTNAACANC
ATATTAAACACACAGAGACCCTACTTCTNACCTNCCTGGNCTTATGAATNNAANCAGCATACCCANNANTCCNCN
NCNACCACTCATACNCTCCTATGAAAAAATNCTNCTACCACTCANCTAGCATTACTTATATGATANGTCTCCA
55 TACCCNNTNNAATCTCATNATTCNCTCTAACCTNANAANAAGCTTAANGGCNAATNGNAACACACTGGC
GNCCNTTNCNTANCGGANCCGAGCNNNTACCNAGCTTGATGCATAGATTNNGTATTCTNTAGGGGTACCTTATAT
AGCTTGGNGTAATNTGGTCATAGCTGNNTCTGTGTNAAATGGCTANACGCTCACAATNNCACACNNTATACNAGC
NCGNANNNTTNCNCCNNAAGCCTGGCGTGCTAATGAGTGAGCTAACTCACATTAATTNCCTTNCNCTCAC
TGNCCTGCTNCCNC

60 SEQ ID NO. 26: NA16-4-F
AACCTTTNANNNTNANNANNNGNANCGGGCTNNGNGANTNAGCNGNGNAANAAGANNATAAN
GNNNGANCGGCATATGAANATNAATCGACCCTANTAGGGCTTCTTGNCNNATGANTNCGACNA

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- GCNNNNNCCCTAGGCCGCTNCTACCATNGCTTACGNCNANTATNANNACACTGGCTACCNCTANTN
CTNGNNNNANTNANATGANNNNNTCTNNATACCNATTNCGANCNTNNTNNNTCNNCCCTAACNNN
CTAGGCNTAANCCTAAGGGCNAATGCACCTGTGTGANAGCCGTNTCTAGCTGGAACCNAGCNANN
NNCNANGNTNGATGNATATATNGAGTATTCTATAGNGGNGCCTAAAGAGCTAGCGCGTATCTNCA
5 TGGNATNCGTGCGCCTNCTGTGANANTGTTNATANCGNNAANAANTGTACAGNCNANTNNAATNAC
NGAANNNTNACAANNNNNGCCGAGGAGGNCCTAANGNGATATGCNNNCTCTTATGNTTNTCCGGC
NCACNTNACTGNCNGCTNTCCGNGCCNGGNA
- 10 SEQ ID NO. 27: NA16-4-H
AAGCTTTTTTTTTTAAAGAGGAAAACCCGGTAATGATGTCGGGGTTGAGGGATAGGAGGAGAATGGGGGATAGGT
GTATGAACATGAGTCAAGCTCAACAGGTCTTCTTTCCCGCTGATTCCGCCAAGCCCGTTCCCTTGGCTGTGGT
TTCGCTGGCTAAGGGCGAATTC
- 15 SEQ ID NO. 28: NA16-4-L
AAGCTTTTTTTTTTAAAGAGGAAAACCCGGTAATGATGTCGGGGTTGAGGGATAGGAGGAGAATGGGGGATAGGT
GTATGAACATGAGGGTGTCTTCTCGTGTGAATGAGGGTTTATGTTGTTAATGTGGTGGTGAGTGAGCCCCATT
GTGTTGTGGTAAATATGTAGAGGGAGTATAGGGCTGTGACTAGTATGTTGAGTCTGTAAAGTAGGAGAGTGATAT
TTGATCAGGAGAACGTGGTTACTAGCACAGAGAGTTCTCCAGTAGGTTAATAGTGGGGGTAAAGCCGAGGTTAG
CGAGGCTTGCTAGAAGTCATCAAAAAGCTATTAGTGGGAGTAGAGTCAAGCTCAACAGGGTCTTCTTTCCCGCT
20 GATTCCGCCAAGCCCGTTCCCTTGGCTGTGGTTTCGCTGGCT
- 25 SEQ ID NO. 29: NA16-4-E
AAGCTTTTTTTTTTATAAGATTATTAGTATAAAAGGGGAGATAGGTAGGAGTAGCCTGGTAAGGGCGATGAGTG
TGGGGAGGAATGGGGTGGGTTTTGTATGTTCAAAGTGTCAATTTATTTTACGTTGTTAGATATGGGGAG
TAGTGTGATTGAGGTGGAGTAGATTAGGCGTAGGTAGACTAGAGTCAAGCTCAACAGGGTCTTCTTTCCC
CGCTGATTCCGCCAAGCCCGTTCCCTTGGCTGTGGTTTCGCTGGCT
- 30 SEQ ID NO. 30: NA16-5-A
AAGCTTTTTTTTTTATAAGGGTGGAGAGGTTAAAGGAGCCACTTATTAGTAATGTTGATAGTAGAATGA
TGGCTAGGGTGACTTCATATGAGATTGTTGGGCTACTGCTCGCAGTGCGCCGATTAGGGCGTAGTTTGA
GTTTGATGCTCACCTGATCAGAGGATTAGTAACGGCTAGGCTAGAGTCAAGCTCAACAGGGTCTTCT
TTCCCGCTGATTCCGCCAAGCCCGTTCCCTTGGCTGTGGTTTCGCTGGCTAAGGGCGAATTC
- 35 SEQ ID NO. 31: NA16-5-G
G AATTCGCCCT TAGCCAGCGA AACACAGCC
101 AAGGGAACGG GCTTGGCGGA ATCAGCGGGA GTCCGAACATA GTCTCAGGCT
151 TCAACATCGA ATACGCCGCA GGCCCTTCG CCTATTCTT CATAGCCGAA
201 TACACAAACA TTATTATAAT AAACACCTN ACCACTACAA TCTTCCTAGG
251 AACACATAT GACGCACTCT NCCCTGAAGT CTACACAACA TATTTTGNCA
40 301 CCAAGACCCT ACTTCTAACC TCCCTGGTCT TATGAATTC
- 45 SEQ ID NO. 32: NA17-1-D,E,F
GAATTCGCC CTTGACCGCT
101 TGTGAATGCA AACAAAATTC AAATTTCCCT GAAAATTTAT TCAACTTCTA
151 TATGCCAAGC AACTGCTAA AGGCTTATCT TCTAAGTATA TGCAGGCATA
201 CCTACTCAC ACAAATAGCT TATTACCAGA GATAGGAAAT TGCAGGTAAT
251 TTGGGAGAAA TTGTCATAGC CAAATTTATG GAAAAAATAA AATAAAAACT
301 TCTCTATGGC CTCTTGATTT AAGAAAAAAA CAGAACAATA CTAAAAAATA
351 AAAGCTTAAG GGCGAATTC
- 50 SEQ ID NO. 33: NA19-1-A,B,C
GAATTCG CCCTTAAGCT
101 TTTTTTTTTT AAGATTGTTC TAATTCTGGT TGTAAGTGC TATTTTAAAA
151 AACAAAACAA ACAGAAAACA TCAAAAACAC AAAAAAGATAT TAAACAGCA
55 201 AGTCTTTTGT ACATCACTGT AGCATAAGCT GCTTGAGGTT GTCATGCAGA
251 ATAGTATCCT TCACGTCACG GAAACAAGG CCGATGTTCT CCGTGTGTGAT
301 AGCAGTGGTG AAGTGGTGGT ATAAGGGCTT CTGTTGCTGG TCCCGACGTT
351 TGAAGGGCGA ATTC
- 60

SEQ ID NO. 34: NA19-2T-C

GAATTCG CCCTTAAGCT TTTTTTTTTT AAGATTGTTT TAATTCTGGT
 151 TGTAAGCTGC TATTTTAAAA AACAAAACAA ACAGAAAACA TCAAAAACAC
 201 AAAAAAGATAT TAAAACAGCA AGTCTTTTGT ACATCACTGT AGCATAAGCT
 251 GCTTGAGGTT GTCATGCAGA ATAGTATCCT TCACGTCACG GAAAACAAGG
 301 CGGATGTTCT CCGTGTGAT AGCAGTGGTG AAGTGGTGGT ATAAGGGCTT
 351 CTGTTGCTGG TCCCAGCTT TGAAGGGCGA ATTC

SEQ ID NO. 35: NA19-2T-A,F

G AATTCGCCCT TCAAACGTCG GGGCATTCCG
 101 GATAGGCCGA GAAAGTGTG TGGGAAGAAA GTTAGATTTA CGCCGATGAA
 151 TATGATAGTG AAATGGATTT TGGCGTAGGT TTGGTCTAGG GTGTAGCCCTG
 201 AGAATAGGGG AAATCAGTGA ATGAAGCCTC CTATGATGGC AAATACAGCT
 251 CCTATTGATA GGACATAGTG GAAGTGAGCT ACAACGTAGT ACGTGTCTGTG
 301 TAGTACGATG TCTAGTGATG AGTTTGCTAA TACAATGCCA GTCCAGGCCAC
 351 CTACGGTGAA AAGAAAGATG AATCCTAGGG CTCAGAGCAC TGCAGCAGAT
 401 CATTTCATAT TAAAAAAGAA GCTTAAGGGC GAATTC

SEQ ID NO. 36: NA19-2T-B

GAATTCGC CCTTAAGCTT TTTTTTTTAT TATGAAATGA TCTGCTGCAG
 151 TGCTCTGAGC CCTAGGATTC ATCTTTCTTT TCACCGTAGG TGGCCTGACT
 201 GGCATTGTAT TAGCAAATC ATCACTAGAC ATCGTACTAC ACGACACGTA
 251 CTACGTTGTA GCTCACTTCC ACTATGTCCT ATCAATAGGA GCTGTATTGT
 301 CCATCATAGG AGGCTTCATT CACTGATTTT CCCTATTCTC AGGCTACACC
 351 CTAGACCAA CCTACGCCAA AATCCATTTT ACTATCATAT TCATCGGCGT
 401 AAATCTAACT TTCTTCCCAC AACACTTTCT CGGCCTATCC GGAATGCCCC
 451 GACGTTTGAA GGGCGAATTC

SEQ ID NO. 37: NA19-2b-A,B,C

GAATTCGCC CTTAAGCTTT TTTTTTTTAA GATTGTTCTA
 151 ATTCTGGTTG TAAACTGCTA TTTTAAAAA CAAAACAAAC AGAAAACATC
 201 AAAAAACACAA AAAGATATTA AAACAGCAAG TCTTTTGTAC ATCACTGTAG
 251 CATAAGCTGC TTGAGGTTGT CATGCAGAAT AGTATCCTTC ACGTCACGGA
 301 AAACAAGGCG GATGTTCTCC GTGTTGATAG CAGTGGTGAA GTGGTGGTAT
 351 AAGGGCTTCT GTTGCTGGTC CCGACGTTTG AAGGGCGAAT TC

SEQ ID NO. 38: NA19-3-A,C

GAATTC
 101 GCCCTTCAAA CGTCGGGGCA TTCCGGATAG GCCGAGAAAG TGTGTGGGA
 151 AGAAAGTTAG ATTTACGCCG ATGAATATGA TAGTGAAATG GATTTTGGCG
 201 TAGGTTTGGT ATAGGGTGTA GCCTGAGAAT AGGGGAAATC AGTGAATGAA
 251 GCCTCCTATG ATGGCAAATA CAGCTCCTAT TGATAGGACA TAGTGGAAGT
 301 GAGCTACAAC GTAGTACGTG TCGTGTAGTA CGATGTCTAG TGATGAGTTT
 351 GCTAAAAAAA AAAAGCTTAA GGGCGAATTC

SEQ ID NO. 39: NA19-3-B

GA ATTCGCCCTT AAGCTTTTTT
 101 TTTTLAGAAT TAAGATTGTT CTAATTCTGG TTGTAACTG CTATTTTAAA
 151 AAACAAAACA AACAGAAAAC ATCAAAAACA CAAAAAGATA TTAAACAGC
 201 AAGTCTTTTG TACATCACTG TAGCATAAGC TGCTTGAGGT TGTCATGCAG
 251 AATAGTATCC TTCACGTCAC GGAAAACAAG GCGGATGTTC TCCGTGTTGA
 301 TAGCAGTGGT GAAGTGGTGG TATAAGGGCT TCTGTTGCTG GTCCCGACGT
 351 TTGAAGGGCG AATTC

SEQ ID NO. 40: NA22-3-B

GA ATTCGCCCTT AAGCTTTTTT
 101 TTTTACTCT CAGGTTTCAGG GTACTAAGTT GAAGTTCTTA CTAGGAAAGA
 151 TGCATATTAA TAATGTATTT GTGGCTTCTT GAGTGCACAG AAGTGATTCT
 201 GACATATGGG CAGGAAAAGT GACATTTCAGG TGAAAACACT ATGGCCAGGG
 251 ATCAAAGGGC GAATTC

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SEQ ID NO. 41: NA22-3-D

5 GAATTCG CCCTTAAGCT
 101 TTTTTTTTTT AAGATAAATG TTGAATTGCA GGAAGAATAA CATTTTGGAA
 151 CAGTAATGTG GGATATAAGA GAAAGTCACA TAGCTCCAAA TTTAGGTGA
 201 GACTTTACAT GTCTTANAAG ACCATTAAGA GGAAGGAACT CAAGTAGGGG
 251 AGACCAAGTT TCAATTAGGG CAGAAGATAG GGAAGGAACT CTATAAGAG
 301 ACTAAAAGT TGAAGGTTCC CTGGCTAAGG GCGAATTC

10 SEQ ID NO. 42: NA22-3-F

GAATTCG CCTTTGATCC
 101 CTGGCACTTG AACACTAATG AATATTATGA CTGCCACTTT AAAGGAGGCA
 151 GAAGAAGTTT AAAAAAGTAA ACAAAAAGTT TGTTCAGAA AACAAGCATT
 201 TTACCTCTGT TTCAAATAGT CTAATTTTTT TAGTGATGAA AACTTCTGAG
 15 251 ACCAGTAGAT GTTTGTAAT AAAAAACATT TATGGCAGTC TTTGTAAGT
 301 TAATGAAACT GGTAGAGAGT AATAATAGCC TGTTTTTGT TTGTTTGT
 351 GCTTTGGGGG ATATTGCAA TACAGTTTAT TGATATGTCA CATACTGTA
 401 AAGTTTATAA TTCACCCAG AATTTATATT ACTAAGTTG TGCTAGTAT
 451 AAAGAGCTTT TCAAATTCAG TGCCTGTATA AAAAAAATA GCTTAAGGGC
 20 501 GAATTC

25 SEQ ID NO. 43: NA22-3-G

GAATTCG CCCTTAAGCT
 101 TTTTTTTTTT ATGTGTTGTC GTGCAGGTAG AGGCTTACTA GAAGTGTGAA
 151 AACGTAGGCT TGGATTAAG CGACAGCGAT TTCTAGGATA GTCAGTAGAA
 201 TTAGAATTGT GAAGATGATA AGTGTAGAGG GAAGGTAAAT GGTGCCAGG
 251 GATCAAAGGG CGAATTC

30 SEQ ID NO. 44: NA22-3-C,E

GAATTCGCTT TTTGATCCCT GGATAGAAAG CCTGAGCCCA TTGGATCTGT
 151 GAAAGCCTCT AGCTTCACTG GTGCAGAAAA TTTTCTCTA GATCAGAATC
 201 TTCAAGATC AGTTAGGTTT CTCAGTCAAA GAAATAAAAT GTCAGGCAGT
 251 GAATGAATTA TATTTTCAGA AGTAAAGCAA AGAAGCTATA ACATGTCGTG
 301 TACAGTACAC TCTGAAAAGA AATCTGAAAC AAGTTATTGT AATGATAAAA
 35 351 ATAATGCACA GGCATGGTTA CTTAATATTT TCTAACAGGA AAAGTCATCC
 401 CTATTTCTCT GTTTTACTGC ACTTAATATT ATTTGGTTGA ATTTGTTTCTC
 451 TATAAGCTCG TTCTTGTGCA AAATTAATAA AATATTTCTC TTACCTTATA
 501 AAAAAAATA GCTTAAGGGC GAATTC

40 SEQ ID NO. 45: NA26-3-A

GAATTCG
 101 CCCTTAAGCT TTTTTTTTTT ACAGATGTGC AGGAATGCTA GGTGTGGTTG
 151 GTTGATGCCG ATTGTAACATA TTATGAGTCC TAGTTGACTT GAAGTGGAGA
 201 AGGCTACGAT TTTTTTGATG TCATTTTGTG TAAGGGCGCA GACTGCTGCG
 45 251 AACAGAGTGG TGATAGCGCC TAAGCATAGT GTTAGAGTTT GGATTAGTGG
 301 GCTATTTTCT GCTAGGGGGT GGAAGCGGAT GAGTAAGAAG ATTCCTGCTA
 351 CAACTATAGT GCTTGAAGTG AGTAGGGCAG AGCAAGGGCG AATTC

50 SEQ ID NO. 46: NA26-3-B,C

GAA TTCGCCCTTT GCTCTGCCCT
 101 AATTGAATTT GCAACTGTTA ATTACTTCAC CAAAAGAGGA TGGGCTTGGG
 151 ATGGGAAGAG TGTAGTAAAT GACAAGTCCC CTTCAATAAA AGCTGAAGGC
 201 ATTACATTAA CATACAACTC AGTGAAAGCA ATTCTTCAAG GAGCTAAGCT
 251 AATATGGTCC AAGTATATAG CTTTCTCATG GCCCAGTTTA TTCCAAGAAA
 55 301 AGACTTTAGA GTACTTAGAG AAGTGGATGG ACTGTTTAAC CTTCAAACAA
 351 TCTTCTAAAA AAAAAAGCT TAAGGGCGAA TTC

60 SEQ ID NO. 47: NC4-1-F

GAATTCG CCTTAATCGG GCTGGAGCTA TTGATTAGCA AGTAAGTAGG
 151 CGTTTGCTAA AACTAGAGAG AGAATTTATG AGGTTATTCA GGGAGAGGAT
 201 ATAGGGTGAT AATTACAATG GACAAAGAAT AGATCTTGAG CTGCACAAAC
 251 ATTTAAGGCA CAGGTAGAAG AAAAGGAGTC TATGTTAAGA GAAGGAATGG

301 TCAGAGAAAC AAGAGGGGGA ACTAGGAGAA AATGGTATTA TGAAAAACAA
 351 AGGAGTAGAA ATTTGAAAAA AAAAAAGCTT AAGGGCGAAT TC

SEQ ID NO. 48: NC4-1-G,H
 5 GAATTC GCCCTTAAGC TTTTTTTTTT TCAACAGCAA CACAGGTTTA
 151 TTACNAGCAA AACCCCTGCGG AGGGGGAAAC CAGCTTAGTG TCAGTGCCCA
 201 CTGCCGCTCA CAGGCTGGGG TAATCATAGC GCTGGGAGGG AGGGCTCTGG
 251 ACAGTATAGC TTGCTGCTCA GTAGAAGATG ATAAGGATGT TCCTGAAGTC
 301 AGGCTGTTGG GCCTTTGCCC AGCAGGATGT GATAAGGATG TTTCTGCAGT
 10 351 CAGGTGGTTA GGACATTTCT CACAGCCCGA TTAAGGGCGA ATTC

SEQ ID NO. 49: NC16-4-G,C,O
 GAATTCG CCCTTAAGCT
 15 101 TTTTTTTTTT CATCCAGTTT TGAAGTAACA TCTTCCTTCC GAACAATCAC
 151 CTGCTTTATT GATGGACGTT CTGTTTCTTT GAATCTTTGA GATCTATATG
 201 CATCAATGCT GTAAAGAAGA TCACGATCTT CAGAACCAAG GCTATCACCA
 251 GATTCAGCTC GAGGGACACG AGTTCTTTGG AATTTTCCTG GTTTTGGACT
 301 TTCAATCATT CTGCTGGTGT CTTTCAATTC CAGTCTAGGT GTGGACACTA
 351 AACTCTCTGG ACTTACCACA CCAACTGTTT GTGCCAATGG TGCAAGTAAA
 20 401 GACATTGAGG AGATATTGAG TGCATCTTCC TGTTCTTCGC TGGCTAAGGG
 451 CGAATTC

SEQ ID NO. 50: NC17-1-A,H
 GAATTCGCC CTGACCGCT TGTGAATAAT ATTGTCTCTA
 25 151 TAGGTGTGCA AGCATTTTCT GGAAGCTATT GAAAACAACA AGTATGGCTG
 201 GTTTTGGGTA TGCCCTGGAG GGGGTGATAT TTGCATGTAT CGTCATGCAC
 251 TTCTCTCTGG ATTTGTGTTG AAAAAAAGCT AGCTTAAGGG CGAATTC

SEQ ID NO. 51: NC17-1-D,G
 GA ATTCGCCCTT GACCGCTTGT
 30 101 ACTGAAGGGA ACAGAGACAG AATGAAATGA AAGAAGGCAG TTGAACTTCT
 151 AGGCTTCTAC AGGCAGAAAA CAGGCTGATA GAACTGCTCA ACTACAGACA
 201 TGTTCTACCT TTCTAGAAAA AAAAAAAGCT TAAGGGCGAA TTC

SEQ ID NO. 52: NC17-2-A
 GAAT TCGCCCTGA CCGCTTGTTA
 35 101 AAAGGAAAAA AGTTGAGAAG TCAGGCCTTG AAAAGAGGAT AGACCAGGCT
 151 GTGGAGGAGT GGAATATTGA GAAGGCTGAG GAACTCAGCA ACCAGCTAGC
 40 201 TACTCGAGAG GAAAATCATG GGAGTAATGT TGTGTGTTTC TCAGTGTGAG
 251 CGATCCAGAG ACTCGTGCTG TCTCTTTGTC CTTTTCCTGA TGGTGTACT
 301 TCGATTAAC TTTTCAAGCTT GGTGTAAAAA TTGCCAAAGC AGTTGCCTGC
 351 CACAACCTTG TAAAAGCCAA AAAGGAGGTT GAAAATTAC AGGCTGCCCG
 401 AAAAAAAGG AGCTTAAGGG CGAATTC

SEQ ID NO. 53: NC17-2-C,F,H
 GA ATTCGCCCTT GACCGNTTGT
 45 101 ACTGAAGGGA ACAGAGACAG AATGAAATGA AAGAAGGCAG TTGAACTTCT
 151 AGGCTTCTAC AGGCAGAAAA CAGGCTGATA GAACTGCTCA ACTACAGACA
 50 201 TGTTCTACCT TTCTAGAAAA AAAAAAAGCT TAAGGGCNAA TTC

SEQ ID NO. 54: NC17-4-A
 GAATTCG
 55 101 CCCTTGACCG CTTGTTAAGA GGAATGATC TCATATATTT GTATCAGAAC
 151 TGTATTTTAT TGTATATTG TATAGTTTGC TCTCCTGCCC CTCTCCTTAA
 201 AACTGAATGG TGCCAATAAT TTGATACTAA TGACTACAAA AAAAGGTAAT
 251 GCCTCATTTA CTAGTATTGT TGTAAAATGA GGAATGTATG TGAATATTCA
 301 GATAACCGAG GATTAACCC TTAAGTGCTG AATCTTTAAA ATTTTAATAT
 351 ATTTTCTTTG AGGGAAATCT TTCTAAAATG TATTACGCAC TTCCCTGCCT
 60 401 TAGTAAACAG AGTATACTGG AAAAAAAGG AGCTTAAGGG CGAATTC

SEQ ID NO. 55: NC17-4-H
 GA ATTCGCCCTT GACCGCTTGT

10 SEQ ID NO. 56: NC17-4-E,F
GAATTC CATTGTGA

20 SEQ ID NO. 57: NC17-5-A,C

[illegible]

45 SEQ ID NO. 60: NC17-6-F

55 SEQ ID NO. 61: NC17-6-D,E,G
GACCGCTTGT

60
SEQ ID NO. 62: NC17-7-A,B,C
GAATTCG CCCTTGACCG

5 101 CTTGTAATTC GAATCTATTT TTGAAGTCGT ATTCTCACAG CATTTCATGCT
 151 TCACAGATGG ACAGATGGAT CCACTTGAGC ACTTTTCTTT GATAAATTGG
 201 ACTAATTTAT CTTAATAATA TGAGGACACC ATCTAAAGGA ACTTTATAAT
 251 TTATCATAAT AAGGAGGTAA CCATACAATA TTTAAAAGAA AATGAATCCT
 301 TTTTCTATTT TAAAGCTCAT TGTCTGAAT GAAATACTAC AGACCTGTAT
 351 TGTAACAAA AAGAAAATGG GGAAAAAAA AAAGCTTAAG GGCGAATTC

SEQ ID NO. 63: NC17-8-A,C,D

10 GAATT
 101 CGCCTTGAC CGCTTGTAAT GAAGGAACA GAGACAGAAT GAAATGAAAG
 151 AAGGCAGTTG AACTTCTAGG CTTCTACAGG CAGAAAACAG GCTGATAGAA
 201 CTGCTCAACT ACAGACATGT TCTACCTTTC TAGAAAAAAA AAAAGCTTAA
 251 GGGCGAATTC

15 SEQ ID NO. 64: NC17-8-E

G AATTCGCCCT TGACCGCTTG TATAATATAT
 101 GTTCCCAGGC AAGAAAATTT TCGTGGTATC AAAGCAAAGT GGAAATCAGA
 151 AAATGTGAAG GTAGTCTAAA TGTCTTGCAA GCAGAAGTTT GGTAGGACCA
 201 GACATACGAT TTAGTTAATG GTCTATTACT TTCCACTGAA AAGCTTGTTT
 251 TATATTAAAA ATGGATCATT TCATTGAAG TACAGTTGGT CCTCTGTATT
 301 CATGGGTTCT GCAGCCAACG ATTCAACCAA CATGGATGGA AAATATTTGA
 351 AAAAAAAA GCTTAAGGGC GAATTC

25 SEQ ID NO. 65: NC17-8-H,I

GA AATTCGCCCT AAGCTTTTTT
 101 TTTTCTGAT TAAGTTACAA ACATTCTCCC TATAGCTAAA CTCCGTGACT
 151 AGGCTCCCAG CCTCATGGCC AAGAACAATA AGTTCACCCA CTTATCTGGA
 201 GTAACCATAC TAGATTAAG AAATACAATT CTTTCTTCTA AAGACAATTT
 251 CCAGAAAGAC CTGCCTTTCC CTATGGGTAC TTGACACTAG GTCCAGCAC
 301 AGGCTAATCG CTGTATGGTT TCTTCGAAGA TTGGCTTTTC TCAGTTTCTT
 351 TCTCTTTGAT ACTGTACAAG CGGTCAAGGG CGAATTC

SEQ ID NO. 66: NC17-9-B,F,G

GAATTC
 35 101 GCCCTTGACC GCTTGTTAAA ATATTTAAGT ACCAGTTAAC TAGCCAGCCA
 151 ACATGGAACG GGTATAAAGA CCCAGTCTCT GCCTTGAAGA CCTACCATCT
 201 AGCAGATGGA GAGGGACATG CTAACAAATA GGGGCGCTAA GTTTTTAGAC
 251 TGCTATGACA GAAGATTTAA CAAAGGACAG TGGGAGAACA AAAAGAAGGG
 301 GTTAAATCTA CCTGGTGGTG GAGTATGTCA GGAAAGACTT CTTCAGATTG
 40 351 GCAATTTGGC CTGAATCTAG AAAAAAAA AGCTTAAGGG CGAATTC

SEQ ID NO. 67: NC17-9-C,E

GAATTCGCC CTTGACCGCT TGTCCAGGAA GGGTTCCATC AATGGTGAGC
 45 151 ACCAGCCTGA ATGCAGAAGC GCTCCAGTAT CTCCAAGGGT ACCTTCAGGC
 201 AGCCAGTGTG AACTGCTTTT AAATGCATT TTTCTAATGG GCTAAACCCA
 251 GATGGTTTCC TAGGAAATCA CAGGCTTCTG AGCACAGCTG CATTAACAACA
 301 AAGGAAGTTC TCCTTTTGAA CTTGTCACGA ATTCCATCTT GTAAAGGATA
 351 TTAATGTGTT CTTTAACCTG AACCTTGAAA AAAAAAAGC TTAAGGGCGA
 401 ATTC

50 SEQ ID NO. 68: NC17-10-A,H

GAATTC
 55 101 GCCCTTGACC GCTTGTAATG AAGGGAACAG AGACAGAATG AAATGAAAGA
 151 AGGCAGTTGA ACTTCTAGGC TTCTACAGGC AGAAAACAGG CTGATAGAAC
 201 TGCTCAACTA CAGACATGTT CTACCTTTCT AGAAAAAAA AAAGCTTAAG
 251 GGGCAATTC

SEQ ID NO. 69: NC17-10-B,C,D

GAATTCGCC CTTGACCGCT
 60 101 TGTGACAGG ATATGGGAGA TGGAAAAGGA AAGGATCTGC ATCTAGTGAT
 151 TGGAAATATA GGAGTGGTGG GGGTTAGTTT CAGATGCCTG TGGGATATT
 201 AATGTCTGT GTTGAGTTGG AACTATGAGT TCTACAGAGG GCAAGATTTA

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251 GGAGTTGGCA CTCCTAAGTG TCAATACATG TGAATAGGAT CGCTTTGGAG
 301 GGTGAGAAGA GGTCTGAGAA CACTACTAGG GAACAGTGAA GGAAAAAAA
 351 AAAGCTTAAG GGCGAATTC

- 5 SEQ ID NO. 70: NC17-11-A,D,F
 GACCGCTTGACTGAAGGGAACAGACAGAATGAAATGAAAGAAGGCAGTTGAACTTCTAGGCTTCTACAGG
 CAGAAAAACAGGCTGATAGAACTGCTCAACTACAGACATGTTCTACCTTTCTAGAAAAAAAAGCTT
- 10 SEQ ID NO. 71: NC17-11-E,H
 GAATTCGCCCTTAAGCTTTTTTTTTTCTATCTGAGGGGGTCCGTAGGGACGAGAAGGGATT
 GACTGTAATGTGCTATGTACGGTGAATGGCTTTATGTACTATGTACTGTTAAGGGTGGGTAGGTTTGTG
 GTATCCTAGTGGGTGAGGGTGGCTTTGGAGTTGCAGTTGATGTGTGATAGTTGAGGGTTGATTGCTGTA
 CTTGCTTGTAGCATGGGGAGGGGTTTGTATGTGGATTGGGTTTTATGTACTACAAGCGGTC
- 15 SEQ ID NO. 72: NC19-1-D
 GAATTCGCCCTTCAAACGTCGGAGCATGGGCATGGTGAATGGCTTCTAGCTGTTGAAGAATGAAGTC
 AAAAGAATGTATTTGGGGATGGAATAGCTGCAATTTGAGTTCATACTTTCTTTAGTTTCATTTTTGCG
 GTCATGTCCCTGTATCCCTGAGGATGAAAACGGAGATACTTTACAAGCTCAAATGCTTAGATAAGG
 GTGAGTTATAAAAAAGATATTCTGCTACAGGAGAAGTAGTATTCATGTTTAATCTGGTCGGACATCACC
 20 TGTTCCTTCCCTTGGGTGACTTTGCTTGAAAAAAAAGCTT
- 25 SEQ ID NO. 73: NC19-2-A,B
 GAATTCGCCCTTCAAACGTCGGGGGAACATCAGGGGAACAAAACCTGGAGAAAGATGCAGGGGGAAGGA
 GAGTAGGAGAAAAGGGAGGAAGAAGAGAGAGAGATAATATGATTGCTTTAAAAACAATTGCCTTTGT
 TTAATACTCAGTAAAGTTCAGAGTTCTTATTCTAAGTTGAGAATTC
- 30 SEQ ID NO. 74: NC19-2-C,E,F,G
 GAATTCGCCCTTCAAACGTCGGGGCATTCGGGATAGGCCGAGAAAGTGTGTGGGAAGAAAGTTAGA
 TTTACGCCGATGAATATGATAGTGAATGGATTTTGGCGTAGGTTTGGTCTAGGGTGTAGCCTGAGAATA
 GGGGAAATCAGTGAATGAAGCCTCCTATGATGGCAAATAGAGCTCCTATTGATAGGACATAGTGAAGTG
 GGCTACAACGTAGTACGTGTCGTGTAGTACGATGTCTAGTGATGAGTTTGCNAAAAAAAAAAGCTT
- 35 SEQ ID NO. 75: NC19-2-D,H
 GAATTCGCCCTTAAGCTTTTTTTTTTTCAGATTGTTCTAATTCTGGTTGTAACTGCTATTTTAAAAACAAAACA
 AACAGAAAACATCAAAAACACAAAAAGATATTAAACAGCAAGTCTTTGTACATCACTGTAGCATAAGCTGCTT
 GAGGTTGTCATGCAGAAATAGTATCCTTCACGTCACGGAAAACAAGGCGGATGTTCTCCGTGTTGATAGCAGTGGT
 GAAGTGGTGGTATAAGGGCTTCTGTTGCTGGTCCCGACGTTTG
- 40 SEQ ID NO. 76: NC19-3-A,B,C
 GAATTCGCCCTTCAAACGTCGGGGCATTCGGGATAGGCCGAGAAAGTGTGTGGGAAGAAAGTTAGATTTACGCC
 GATGAATATGATAGTGAATGGATTTTGGCGTAGGTTTGGTCTAGGGTGTAGCCTGAGAATAGGGGAAATCAGTG
 AATGAAGCCTCCTATGATGGCAAATACAGCTCCTATTGATAGGACATAGTGAAGTGAGCTACAACGTAGTACGT
 45 GTCGTGTAGTACGATGTCTAGTGATGAGTTTGAATAAAAAAAGCTTAAGGGCGAATTC

- 5 SEQ ID NO. 77: NC19-4-A,B,C
GAATTCGCCCTTCAAACGTCGGGGCATTCGGGATAGGCCGAGAAAGTGTGTGGGAAGAAAGTTAGATTTACGCC
GATGAATATGATAGTGAAATGGATTTGGCGTAGGTTTGGTCTAGGGTGTAGCCTGAGAAATAGGGGAAAT
CAGTGAATGAAGCCTCCTATGATGGCAAATACAGCTCCTATTGATAGGACATAGTGGAAGTGAGCTACAA
CGTAGTACGTGTCGAAAAAAAAGCTT
- 10 SEQ ID NO. 78: NC19-5-B
GAATTCGCCCTTCAAACGTCGGGGCATTCGGGATAGGCTGAGAAAGTGTGTGGGAAGAAAGTTAGATTT
ACGCCGATGAATATGATAGTGAAATGGATTTGGCGTAGGTTTGGTCTAGGGTGTAGCCTGAGAAATAGGG
GAAATCAGTGAATGAAGCCTCCTATGATGGCAAATACAGCTCCTATTGATAGGACATAGTGGAAGTGAGC
TACAACGTAGTACGTGAAAAAAAAAAGCTT
- 15 SEQ ID NO. 79: NC19-5-D
GAATTCGCCCTTCAAACGTCGGCAGGAACCTGCTCGACTGAGAGACTCAGCCTCCAGAGTAGTTGGG
ATTACAGACACGCCACCACCGCGCCCGCCATCATGACTTTTCTCTGCTTCTTGAGAGCACTCCAGCATC
GCTAGTCGCACCTTGTGACTCTCACAGAAGGAGGAAGAGGAGGACACTTTTATTGAAGAACAACAACCTAG
AAGAAGAGAAGCTATTGGAAAAAAAAGCTT
- 20 SEQ ID NO. 80: NC24-1-A,B,C
GAATTCGCCCTTAANCTTTNTNTTTTCAAGANGAGCTGTNTNGTANNATGCTNAGCTGTNTGATAGGNCNAC
CANGTCATANNTNAGGTTNGCCATGGNCNNACTACTNGGACCCAACATGAAATATGACNNNCNNTTNGG
CATAAAGAGGCACACGGGAACATCTGATGGANTAAAAATACTATTATTAATGCNACTACTAATATGA
ATATCTTATTACACAAACAGGAAGAATTACGTATTTACAGGGTATTGGTGAGCAGTCAAAAAGCGTGGC
25 AAATTACCTAAAANGTTTANAAGGTTTAAAGTGATCAAATATTGCATNANATATAATTNCCCCCNNTAAA
GAACCTTGTATTAAATGTGTTTACTATAAGCACAGAATTAACCTTTGCTCTCCTGNANGTACCCANN
TTTGNNCATAACAGAAGANGCATGGGCCTATCTCATACGTATGCNCATACNAACACACATTACAAACANG
GAAAAACGAATGCTAAAGTCTAAAGTACTCCANNCCNANGGCGAATTC
- 30 SEQ ID NO. 81: NC26-1-A,B,C
GAATTCGCCCTTTGCTCTGCCCTACTTAATCACTAACACATCTTATACTGTCTAACCTCCAGAATTT
TGTTGAGATTCTCTGCTGATGTGTTTCTTTGTTTCTCTCTATCACTTAGAGTTTGTGTTT
AATACCTTTGCTATCATTTTATTGTGTTTGGTGGGAGAGGAAATAAATGGCCAACTCCACTACCTCG
35 AAAAAAAAAGCTTAAGGGCGAATTC
- 40 SEQ ID NO. 82: NC26-1*-A,B,C
TGCTCTGCCCTACTTAATCACTAACACATCTTATACTGTCTAACCTCCAGAATTTTGTGAGATTCTCTGCTGAT
G TGTTTCTTTGTTTCTCTCTATCACTTAGAGTTTGTGTTTAAATACCTTTGCTATCATTTTA
TTGTGTTTGGTGGGAGAGGAAATAAATGGCCAATCCACTACCTCGAAAAAAAAGCTTAAGGGCGAATTC
- 45 SEQ ID NO. 83: NC34-1-A,B,C
GAATTCGCCCTTTCCGCTCTGGGGATATCAAACTCTCTAGGTCCAGGTTCAAAATCTTCCACACATTCTCTGTG
TCTGCTTTTAGCCAGACACCATCACTATGTGGTAGCTTACCTCAAAGCTTCACTTAGTGATCAACCTC
AGAAGCACTTTCTGATCCCTTCAACCTGCACATCTGCTTCTTATCTAAATTCCAGCCAGCTCAATCCA
TACCTTCTGACCATGCTACGAAAAAAAAGCTTAAGGGCGAATTC
- 50 SEQ ID NO. 84: NG2-1-C,F,G
GAATTCGCCCTTTGCCGAGCTGGGGAGTATAAAATGTTACCTCATTGTGGTTTCATTTTGTAATTT
TTGATTATTAGTAAGTTGAACATGTTTTATTATGGGAATTCCTGTTTCTTTTCTTGGCAATCCCTCTTC
ATGCTTTTGGCTTTCTTCTTCTATTGAGTTGTGTTTAAATGATTAATTTTAAAGTTCTTTATATTAAAT
ATTTAATTGGTCGACATTTATTTAATCATTAAAGTGAAGAGAAACCAGATTAGAGTAGA
AAACTTTTCTGAGGCCATTCCGGAATATGCTAAGCATGTGAATCTTATTCTATTGGAGAAAATAAA
55 GTTAAATACATATATAAAAAAAAAGCTTAAGGGCGAAT
- 60 SEQ ID NO. 85: NG2-1-D
GAATTCGCCCTTAAGCTTTTTTTTTTTTACCAAATATATTTACTGTTTACAGTAGTAAAGGACAACAAATAA
TGTAACAAATTGGTGAATAACACAACAGAGCCAACAAACATCCACCCGAGCCCATACAGCAAACAGGAA
ATGAGAACATTTTCAAGCAAGATTTCAAGCAAGAGATGATGGGTCTTGTTCAGGTGACTGTAAAAAG
GCAGAGAAATGGCCACCAAGCAGCAATGGAGCCTCAGGGAAGGACAATAGGCAGAACTATGAAAAATGTTTA
ATTGGTATAGATCCCAAATATTTACAGAAGTGAATCACCAGACTAATGCATAAATCAATACCTATT
TGGAAAGCAGCTCGGCA

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- 5 SEQ ID NO. 86: NG2-2-I
GAATTCGCCCTTTGCCGAGCTGTTGTATATTGAGGTGTATTATTTACGTCTCTGGTCCAGTCTTTTCT
GGCAAATAACAGTAAAGATGGTTTAGCAGGTACCTAGTTGGGTCAGAAGATCGATGATCACCAAGCAG
GAAAGGGAGGGAATAGAGGAATGTGTTCCGGGTTAAGTGATGAAATGGCAGTGGTGGCCGGCGTGGTGG
CTCTCGCCTGTAATCTCAGCACTTTGGGAGGCCGAGGCAGGTGGATCACCTGAGGTGAGGAGTTCAAGAC
TAGCCTGGCCAAACATCATGAAACCCCGTCTCTACTAAAAATACAAAAATTAGCCAGGCATGGTGGCACAC
ACCTGTAGTCCCAGCTACTCGGGAGCCCAACGCACGAGAACCCTTGTACCCAGGAGGTGGAGGTTGCAG
TGAGCCGAAGTTGCACCATTCGACTCCACCCTGGGCGACAGAGCAAGATTCTATCAAAAAAAAAAAGCTT
- 10 SEQ ID NO. 87: NG2-2-M
GAATTCGCCCTTTGCCGAGCTGGAAAAGACAATTGATGACCTGGAAGAGCGTCTCTACAGCCAACCTTGAGCGAAA
C CGCCTGCTTTCTAATGAGCTGAAGCTAACGCTGCATGATCTGTGTACTGATGGGCAGGGCTCAATGATG
CCCATTAACCTGAGCTTACTGCTCACACCACTGACCTGGACCCCAACAAAAAGCTGATTGTCTTTTAA
15 AGTTATTATTTGCCCTGAGCAAAATGCATTTAATTGGGGCAGTTAGAATGTTGATTTCTTAACAGCAT
TGTAAGTTGACCATTGTGAAGTTTCTGTCCCTTTAGAAAGAGATTATGGGTGAAGAAGGGAGGGCCTGA
GAGATTATAGTGAGAAAACCTGCGAGAATTTTGTTCACCCCTTATTGTCTGCTCTTTCCTTGGGCAC
TGACTGTAGGATATGTTCCCTTGCATGGATGTTTTTAACAATAAAAGGACTGACTTGACAAAAA
GCTT
- 20 SEQ ID NO. 88: NG2-2-N
GAATTCGCCCTTAAGCTTTTTTTTTTTTGGGAATCTACTGCGAGCACAGCAGGTGAGCAACAAGTTTATT
TTGCAGCTAGCAAGGTAACAGGGTAGGGCATGGTTACATGTTTAGGTCAACTTCCTTTGTCTGTTGAT
TGGTTTGTCTTTATGGCGGGGGTGGGGTAGGGGAAAGCGAAGCAGAAGTAACATGGAGTGGGTGCAGCC
25 TCCCTGTAGAACCTGGTTACGAGAGCTTGGGGCAGTTCACCTGGTCTGTGACCGTCATTTCTTGACATC
AATGTTATTAGAAGTCAGGATATTTTTTAGAGAGTCCACTGTTTCTGGAGGGAGATTAGGTTTCTTGCC
AAGATCCAAGCAAAATCCACGTGAAAAAGTTGGATGATGCAGGTACAGGAATACACAGGGCATAGTTCT
CATAGTCGGTGGCCAGGATCCAGTACGGTGGCGATGGCATAAACAGGAAACTTAACCTCCAGCTCGGC
A
- 30 SEQ ID NO. 89: NG9-1-F,H
GAATTCGCCCTTAAGCTTTTTTTTTTTTGGGCAGTGAGAGTGAGTAGTAGAATGTTTAGTGAGCCTAGGGTGT
GTGAGTGTAATTAGTGCGATGAGTAGGGGAAGGGAGCCTACTAGGGTGTAGAATAGGAAGTATGTGCCT
GCGTTACAGGCGTTCTGGCTGGTTGCCTCATCGGGTGATAGCCAAGGTGGGATAGTGTGGTTTCGA
35 AGAAGATATAAAATATGATTAGTTCTGTGGCTGTGAATGTTATAATTAAGGAGATTGTAGGGAGATTAG
TATAGAGAGGTAGAGTTTTTTTCGTGATAGTGTTCACTAGATAAGTGCGGTTACCCAAGGGCGAATTC

5 CAATCTCTGACCAGAGCCAAGGGCTCCTCTAATGATTGCATCTGTAAATTAGAAGGGAATCTCTGTACT
ATAGGTATTGGTCATATCCTGAAGGAAGTCAAACATGCCGAGTCACACCTTTTTAACTCATGTCTTGAG
GAGAATATAGAGAATTGGTTACAACCTGATTGGATACACTGTATTACAGGTTAAGAATAATTTTCAGT
AAAATTAGAACAGGAAGTCTCTAGACTTTTGAAGTAATGTCTATCCAAAAAAGCTTAAGGGCGAAT
TC

15 GAATTCGCCCTTGTGATCGCAGAGCAAGAAATAACCAACGTCAGAGCTTAACTGAAGAAGGAAATTTGAG
ACTCAGAAAATTATCCAAAAGTTCCAAACATCCAGGGGTTGATTTTTTGAAAAATTTAATAAGATAGATA
GACCACCTAGCTAGACTAATAGAGAAGAGAGACCAATCCAAATAAACACCATTTAGAAATGATGAAGGCAATG
TGACCCTGATCCCACAGAAATACAAGTAACATGAGTAACCTACTAAAAATGCTTCTATGCACACAAACTA
GAAAACCTTCTTAGAAGAGATAGGTAAATTTTCTACACATACACCCTCCCAGACTGAATCAAGAAGTA
ATTGAATGTTTTAACAGACCAATGATGAGCTCCAAAGTTGAATAGGAATAAATAAGCTACCAAAAAAAA
AAGCTTAAGGGCGAATTC

20 SEQ ID NO. 92: NG10-1-B,D
GAATTCGCCCTTAAGCTTTTTTTTTTGGCAGATGGAGCTTGTATAATTATGCCTCATAGGGATAGTACAAGGA
AGGGGTAGGCTATGTGTTTTGTCCAGGGGGTTGAGAATGAGTGTGAGGCGTATTATACCATAGCCGCTAGTTTAA
GAGTACTCGGGCAAGTACTATTGACCCAGCGATGGGGGCTTCGACATGGGCTTTAGGGAGTCATAAGTGGAGTCC
25 GTAAAGAGTATCTTTACTATAAAGGCTATTGTGTAAGCTAGTCATATTAAGTTGTTGGCTCAGGAGTTTGATAG
TTCTTGGGCAGTGAGAGTGAGTAGTAAGTGTTTAGTGAGCCTAGGGTGTGTGAGTGTAAATTAAGTGGCATGAG
TAGGGGAAGGGAGCCTACTAGGGTGTAGAATAGGAAGTATGTGCCTGCGATCCAAGGGCGAATTC

30 GAATTTCGCCCTTTGCGCCCTTCTGATCTATTTCCATCTTTATCTCTCTGTTCTGTTCCTGGGAGGCTGAC
AAATAAAGAAGTCGATACTCTCTTTTCTGGCTTTATTTCATGGTGGGTTTGACAGTGATAGAAAAGTGAGGCTGA
AATATTTGCTCCTTCTTTCTCATATACTTGGCAAGGTCCAAGGGCGAATTC

	GAATTC	NO. 94:	NG25-1-M				
35	101	GCCCTTTGCG	CCCTTCCCCC	AAATGTAGAG	TAAAAATCAT	ACTGAGGGAG	
	151	TTCAGGTTGT	TGCTCAGTGG	TTAACGAATC	TGATTAGCAT	CCATAGGATG	
	201	CAGGCTGCGC	CCCTGGCCTT	GCTCAGTGGG	TCAAGGATCT	GGCGTTGCCG	
	251	TGAGCTGTGG	TGTAGGTTGC	AGATGTGGCT	CAGATACTGC	ATTATTGTGG	
	301	CTGTGGTGTG	GGCTGGCAGC	TATAGCTCTG	ATTCAACCCC	CATCTGGGA	
40	351	ACCTCCATAT	GCCTTGGGTG	CCCTAAAAAA	AAAATCATAC	TTCAGAACCC	
	401	TCATGATCCT	GATGTTCCCT	CAAGAACAGT	CCTCTATGAG	TTCTGCTGT	
	451	GGCACAGTGG	GTTAAGAATC	TGATTGCAGC	GACTCGGGTT	GCTGCTGCAG	
	501	AGGCAAGAGT	TTGATCCTCA	GCCTGGTATA	GTGAATTAAA	GGATCCAGCA	
	551	TTGCCACAGC	CATGGGGTAG	GTTACATCTG	TGGCTCAGAT	TCCGTCTCTG	
45	601	GCTTGGGAAC	CTCCATATAC	TTGGCAAGGT	CCAAGGGCGA	ATTCCAGCAC	
	651	ACTGGCGGCC	GTTACTANTG	GATCCGANCT	CGGTACCAAG	CTTGATGCAT	
	701	AACTTGAGTA	TTCTATAGNG	GCACCTAAAT	AGCTTGGCGT	AA	

50
55

SEQ ID NO. 95: O A2-1-A,C,L,N 2E4
GAATTCGCCCTTAAGCTTTTTTTTTTACACTGTCTAAAGCATTTAATGGTCTTTCTTTAACACAGCCAACTCCC
CCGGGTTTGAACAGCTGTTAAATCTCTCTTGCTGTGGCAAAGAGCTGTCAAGTCCAACACTGAAAAATTGG
TACCAATTTCTGGCCAGTAAGCACAGAACAGAGGGCTAATATTTTATGGTTTTATTATTACTGTGTTCTCA
TGCTGTGTTTTCTTTCTCTGTCTCTCCCTCCTGCTCGTGTCTGCCAGGGCTGATTGTTGTGACATTTGGCCGT
ATGCTGGATGCCCAACAGATCTGGAGGATCATGGCTCGCGCCAAACCAAGCACAGCTGGACAGGTCCTACTT
CCGGGCGTACATGATCCTCCTCCCTTCTCGGAGACGTTTTTCTACCTCAGCTCGGCAAGGGCGAATTC

SEQ ID NO. 96: O A2-1-A,C,L,N 2E4

60 SEQ ID NO. 96: OA2-1-M 2E3
GAATTCGCCCTTAAGCTTTTTTTTTTTTACAGAAGGTCAAACAAATGTATTATATGATTCAAATGGGACTATACAT
CTATTTCATTTTTTAAGAGATAGGAGTGAACAAATGAAAAAATCAACAAAGTACGTGCTTCTATAAATGAAGATA
ATTCCCAAGTTAAGCTACTATATAGTAAGAAATACCATATGCAAACCTTCTAGACCCACAAAAATGGGGAATTT
TTTATCAAACCTTATTA AAAAATAGCATT CATATCAATGTTGCATAAAATACAGGAAAAATATACAACCCCAATAGAAA

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TGTGACTAATGAGTATAAACACACTATGAATAGAATAACAACTCTAAACAGATTTAAGAGCACATATTCTCAACA
TCGTAAGTAGTTGGGGAAATGCAAATTAATAAAACATTTTTCATCTAGCAGCTCGGCAAAGGGCGAATTC

5 SEQ ID NO. 97: OA3-4-1-G 2G7
GAATTCGCCCTTGACCGCTTGTAAGAGGAAGTATCTCATATATTTGTATCAGAACTGTATTTTATGTTATAT
TGTATAGTTTGTCTCTCTGCCCTCTCCTTAAAACTGAATGGTGCCAATAATTTGATACTAATGACTACAAAAA
AGGTAATGCCTCATTTACTAGTATGTTGTAAATGAGGAATGTATGTGAATATTCAGATAACCGAGGATTAAAC
CTTTAAGTGTGAATCTTTAAATTTTAATATATTTTTTTTGGAGGAATCTTTCTAAATGTATTACGCACT
CCCTGCCTTAGTAAACAGAGTATACTGGAGAGTATTTAACCTTTTCTTGATGAGTCATGGTCATGATTATAACA
10 TCAGCCCCCTTTAAAAAAAAGCTTAAGGGCGAATTC

15 SEQ ID NO. 98: OA10-1-B 2F1
GAATTCGCCCTTGATCGCAGGAATCAGGGGAAAGTGATTTTAAAGGTGGTTTCTCCAGCACATTTAAGAAAA
GGGACCAAAAGTTATTTTAGCTTCTCAATAGATTGCATGTTGCTTATTAGGATAATAAATTAATATTAAATGCA
ATATATGTCTGTCTTTATTATGGCATCTATTTAGGAGTTGTTCAAATCACTGCAGTAGGGCTCTGCAATAAAA
TAATGTAACCTATTATCATGGATCTAATGTACTGTAACCTTTATCAGTGAAAGGTAAATCTCAAATAACAAGTAC
AAACATTGAACAATTACCTATAAAGATTTGTAAGTAAATTTTCCAATAGATTTCATTCTGTCTATTTGTA
AGACGACCTGCAGTCCACCTGTTGTAACCTTTTAAATAAATAGACATCTGTAAAAAAAAGCTTAAGGGC
GAATTC

20 SEQ ID NO. 99: OA10-1-E 2F2
GAATTCGCCCTTGATCGCAGGCTGTAAGTCTTTAGCATCTCAGGAAGTTACTAACTTCAAACCTAAGTATATAG
GTAGAGTTTCTTACTAAATCTAGTGCTTCTGAACCACAAGTAGAAAGCATTTAAACATGAATGTTGTTTGTG
TTTTTTGAAGTTTGTAAATAGAAGACTTGTGATGATCCGATGGCAAGGTATTTTCTCTTGGTATGATTTTAG
25 TTTATTTCCCTCGTGATGCATAAGTGAAAGAGTGAAGTTTCTCAGAATGAGCAACTGTCTCATCTACCTGCTAT
TTTATTATTGCTGATTACAAAAGCAAATCAAGAGATGAGAACCCAGTTGCTGCAAGTAAATATTTACTGCATTG
AGGGTCGGAGCATTTTCCATTACCGTTATCCATGGATCAAAATAGTGATCTCAGTGGTAATTTAGAGGGCCA
TTAAACCCCTGATGGTGCTGGAAGAGATGGCAGTGCTCATGTGCAAAATAGGTAACTGTAATTAAGAAGTTAC
AGATGATTTGATTACGCTCTTGNGTATTTGGTCTGTATAATGTGAGCAGATTAAAAATCATGTAAGTGCTTAA
30 AAAAAAAGCTTAAGGGCGAATTC

35 SEQ ID NO. 100: OA10-1-H 2E12
GAATTCGCCCTTGATCGCAGTATTCCTGTGATGGAAGTCATCAGATATGCTGTGCAAGTCTTGCTTAATGTAT
CTAAGTATGAGAACTACTTTCAGCAGTTTATGATGTAGAAAATTTGATAGATATACTATTGGAGCTTTTGCAGA
TATGCCGAGAAAAGCCTGGTAATAAAGTTGCAGACAAAGGCGGAAGCATTTTACAAAACTGTTGTTGTTGTTG
CTATTTTACTGAAGACAACAAATAGAGCCTCTGATGTACGAAGTAGGTCCAAAGTTGTTGACCGTATTTACAGTC
TCTACAACTTACAGCTCATAAACATAAAATGAATACTGAAAGAATACTTTACAAGCAAAGAAGAATTCTCTA
TAAGCATTCCTTTATCCCAGAAACCTGTAAAGACCAGAATAGTTTCAAGACTTAAGCCAGATTGGGTTTGA
GAAGAGATAACATGGAAGAATCACAATCCCTGCAAGCTATTCAAATGGTGATGGATACGCTTGGCATTCCCT
40 ATTAGTAAATGTAAACATTTTCAGTATGTATAGTGNAAAGAAATATTAAAGCCAATCATGAGTACGTAAAAAAA
AAGCTTAAGGGCGAATTC

SEQ ID NO. 101: 10A17-1-A,B,C
GAATTCG
45 101 CCCTTAAGCT TTTTTTTTTT AATGGTTGAA GTAAGTGAAG ATATTTAATC
151 TAGAGAAGAT TTGGGAAACA CATGATAGCT ATGGTTAAAT ACTTAACAGG
201 GCAATCACAG GGAAGATGAC TAGATTTCCT AACATCCATG AGTGAAATTT
251 ATAGAAGTAT ACTCTCTGAC TTGATATAAA GGAAGATTTT AAAAAACATG
301 ACTGTTTCAGG AGTGTTCAAG TAGGGTCAGA TGACCACTGA TTGGGAATAC
50 351 TTCGTAAGCA GGAGCAAGTA AGATCTGAGC CACTGTTCTA TCGGTAGGGT
401 GTCTGTGGTA TTCCTTGGTC AAAGAAGTAC TCTAAGCAAC TTCAGTCTCA
451 CGAATTACTA TCACCTCGT GGCATACAT GATGGTTACC CTAAAGAGGA
501 AGTTTCAGAA GGCAGTAATA TGGATCCTG GAATAGTCAG ACAGGAGCCT
551 TCATGCAGAT ACCCTTTTCA GTTCTCCATA CACCCATTCA CAAGCGGTCA
55 601 AGGGCGAATT C

SEQ ID NO. 102: OA17-2-A,B
GAATTCG CCCTTGACCG
60 101 CTTGTGAGGA GGAAAGTAAT GCTGGGAAAC TTGATATGTG TAAATAGAAA
151 ATATATAAGC AAAGTTATCA GCCAGTCTTG ATGTTGCAGC GGAAGTTGAG
201 AGTGCCGTGG TATATCCTGT TTTGTGCATT AGCTTTTCT GGGGCATGAG
251 CATTCAAGCA TTTTATGAAG AACTTAGAAA AAGTAAAAA TATTTTGAAG

301 TTTTATATCT TTGATCATT GCTGGAAGGT TTGTCCAGTA GTAAGTTACT
 351 TGTGAGGTTT ATAAAATATT AGGAACATTT GGCAAGAAGA GACAGGTTTT
 401 GTGGGAATAA TTTGTTACCT GTTGACCCTC ACTGTGGACA TATTGTGTG
 451 TGTGTACCTG TGTGTGTGTG TGTGTGTGTG TGTGTGTAAA AAGGAGGGTT
 501 TATAAAAAA AAAAGCTTAA GGGCGAATTC

SEQ ID NO. 103: OA17-3-A,B,C
 GA ATTCGCCCTT AAGCTTTTTT

101 TTTTATACT GCCTTGGAAAT ATTATCAGAT GGATAAATTA GCAGCACTTA
 151 CTCCGCTGCA TAAAACAAAA TAAAATCATT TGACCTAGGC AAAAACTTA
 201 ATTTTGAAAT ACTATGTGTT TATTATCTCT AACAGCTCTA ATATTCCTT
 251 CTCAATAAAA TTTAAATTTT AAAATAAGCA TTAAATAACT AGCATTCAAT
 301 GCCAACACCA GTTCAGCATA TTCATTAAAA CCTTATTACA TTCTGATACT
 351 GGTATTGGTG ACTTCACTTG ATAATAAACT GTTGCTCAGA AAGGCCAAGG
 401 ATTCTGTCCA AAGTCCCAGA GCTGGGAGCA GCTGAATTAG GAGTTGCCCT
 451 GTCTGATTAT GTAGCCGGGG ATGTTTTTCAG TCACAAGCGG TCAAGGGCGA
 501 ATTC

SEQ ID NO. 104: OA17-5-B 2F4

GAATTCGCCCTTAAGCTTTTTTTTTTTTAGCAGTAGAAATAAACCTAATTACTCATAAACCATATTTGAAATGAG
 AATAAATCAACAGCTTCATTTTGGAGCCTTTTAGAGTGTCTAGAATATCTGGCCAAGGTAGACTGTGAAAGGTAGG
 CTCTTCTTTAAACACGGTTATGGTTCAGCAGTTATTTGCAGGCTCTTGGGAAGGCACTGTGCTGAAGGAGAGCAA
 AGAGTTTCTTTTGTGCTTTTTTTTTTTTGGAGNGGAAATATTTCTTGTGACAGTCAAGGCTCTTTTCTGATTT
 TTTGTGCTCATACAAGCGGTCAAGGGCGAATTC

SEQ ID NO. 105: OA17-5-E,F,G 2F9

GAATTCGCCCTTGACCGCTTGTGGCAACTTAGTGGAGTATGTTCCCTCTCAGGTAATATACAGAGAAGACAGGTT
 AGAGGGTCTGTCTGTGAGTGTATGAATTCCTTTTAGATTGGATGACTGATTTTCTTACTTTAGTAAAGTTTCA
 AGTGCATGTGGACTGAAGGGCAGTAAGGAGGGCAGTAACAGCTATGGGAATTCCTAAAGAAATTCATCAGAGATG
 AATGTAACGATTATGGAGTGAAGTATTTGAAATTTTGAAGTTAGCAGGGTTTGTACTGTGCCAGTCTTTCATGA
 TTTAAAAAAGCTTAAGGGCGAATTC

SEQ ID NO. 106: OA17-5-H,J 2F5

GAATTCGCCCTTGACCGCTTGTGATGTCTACGGAAAGTGTGCTAGAAATTTAGTTAGGATTGTGTTGTGTCTAT
 AGATCAAATTGTGAAGAAGTCACATCTTAGCAATACCTTAGTTTGTATTCCATGAACACAATATAATCTAATCAG
 CACATAGATCCTGTACATATCTTACTGGATTTATACCCAAGTATGAAGTGTCTGTTTCTGGTTTTTTTTTGTGTGTG
 GGGGTGGGGGACATGTGCTATTTGAAATGGTATATTCAATTGTTTCTGCTGATATGGAATAAATTTGGCT
 TCTGTAAAAAAGCTTAAGGGCGAATTC

SEQ ID NO. 107: OA19-1-B,D,G

G AATTCGCCCT TAAGCTTTTT TTTTAAAGA TTGTTCTAAT
 151 TCTGGTTGTA AACTGCTATT TTAATAAACA AAACAAACAG AAAACATCAA
 201 AAACACAAAA AGATATTAAA ACAGCAAGTC TTTTGTACAT CACTGTAGCA
 251 TAAGCTGCTT GAGGTTGTCA TGCAGAATAG TATCCTTCAC GTCACGGAAG
 301 ACAAGGCGGA TGTCTCCGT GTTGATAGCA GTGGTGAAGT GGTGTATAA
 351 GGCTTCTGT TGCTGGTCCC GACGTTTGAA GGGCGAATTC

SEQ ID NO. 108: OA19-2-B,E

G AATTCGCCCT TAAGCTTTTT TTTTAAAGAT
 101 TGTCTAATT CTGGTTGTAA ACTGCTATTT TAAAAACAA AACAAACAGA
 151 AAACATCAAA AACACAAAAA GATATTAAAA CAGCAAGTCT TTTGTACATC
 201 ACTGTAGCAT AAGCTGCTTG AGGTTGTCTAT GCAGAAATAGT ATCCTTCACG
 251 TCACGGAATA CAAGGCGGAT GTTCTCCGTG TTGATAGCAG TGGTGAAGTG
 301 GTGGTATAAG GGCTTCTGTT GCTGGTCCCG ACGTTTGAAG GGGCGAATTC

SEQ ID NO. 109: OA19-3-D,E

GAATTCG CCTTAAGCT
 101 TTTTTTTTTT AAGATTGTTC TAATTCTGGT TGTAAGTGC TATTTTAAAA
 151 AACAAACAAA ACAGAAAAA TCAAAAACAC AAAAAGATAT TAAACAGCA
 201 AGTCTTTTGT ACATCACTGT AGCATAAGCT GCTTGAGGTT GTCATGCAGA
 251 ATAGTATCCT TCACGTCACG GAAAACAAGG CGGATGTTCT CCGTGTGTGAT
 301 AGCAGTGGTG AAGTGGTGGT ATAAGGGCTT CTGTTGCTGG TCCCGACGTT

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351 TGAAGGGCGA ATTC

SEQ ID NO. 110: OA19-5-D,F,G

5
101 TTTTAAAGCT
151 TTTTAAAGCT
201 TTTTAAAGCT
251 TTTTAAAGCT
301 TTTTAAAGCT
351 TGAAGGGCGA ATTC

15
SEQ ID NO. 111: OA34-1-A 2G2
GAATTCGCCCTTAAGCTTTTTTTTTTAAAGAAAAGATCTGCCCATCACCTATATTTTTATTTATCTCATGGGAT
TTTCGTATTTTCTGGGAATGCAGGCACTCTGTTCTTATCATGGCTGAAATACGGTAGGCTTAATACTTCACAAT
TATATAGCACCTTACCCCAAGGGCTGTTGTTGGTTTATGTGTGTTTAAATCAGCTTCCAGAATTGC
CATGCCCTCACCTGTGAAGTGGGATAGGCAGGGTCCCCAAGAGGTGATCACTCCAGGTGGTGTCTAAGCCAGAGCG
GAAAGGGCGAATTC

20
SEQ ID NO. 112: OA34-1-B,C 2G4
GAATTCGCCCTTTCCGCTCTGGGACTATTACATTTAATTCTGCTCTTGATAGTCAAAGACCATGGACAACAACCTG
TCATCTGAAGGACTCTCTAGAAAGCAATTTATCCATTTAAGCACTTACACAGTTAGTCATGGAGAGTAACAGGCCTGCT
TGGGGTTATGTAGAAAAGCAATTTATCCATTTAAGCACTTACACAGTTAGTCATGGAGAGTAACAGGCCTGCT
GGTGAACAGGTACCCCAAAATGGAGATGGCATCAACTAGTGGTCAAGGACTAACTCCTAAAAAAAAGCTTA
AGGGCGAATTC

25
SEQ ID NO. 113: OA34-1-F,H 2G6
GAATTCGCCCTTTGCTGTTGAAGAAGGCATTGTTTTGGGAGGGGTTGTGCCCTCCTTCGATGCATTCCAGCCTT
GGACTCATTGACTCCAGCTAATGAAGATCAAAAATTTGGTATAGAAATTATTAAGAAGCACTCAAAATTCAGC
AATGACCATTGCTAAGAATGCAGGTGTTGAAGGATCTTGATAGTTGAGAAAATTATGCAGGTTCTCAGAGT
30 TGGTTATGATGCTATGGCTGGAGATTTTGTGAATATGGTGGAAAAGGAATCATTGACCCAACAAGGTTGTGAG
AACTGCTTTATGGATGCTGCTGAAGGGCGAATTC

35
SEQ ID NO. 114: OC13-1-A
GAATTCGCCCTTGACCGCTTGTGAATAATATTGTCTCTATAGGTGTGCAAGCATTTCTGGAAGCTATTGAAAAAC
AACAAGTATGGCTGTTTGGGTATGCCCTGGAGGGGTGATATTTGCATGTATCGTCATGCACTTCCTCCTGGA
TTTGTGTTGAAAAAAAAGCTTAAGGGCGAATTC

SEQ ID NO. 115: OC13-1-C,D

40
GAATTCG CCCTTCAGCA
101 CCCACTGAAA AACAAGTTGA GTAGAGAGTG TAGAGTGCAG AAATGTGGCT
151 TTTGCCCCAC TTGTCATCTC CAAAATTACA ACGGTTGGCC GATCCCATTT
45 201 GAGGACAATG CTTAGTTATA AGTCTCCGAG TTGAAAAGG AAGAAAGCCA
251 GAGCTGTCTA GTTTCATTCA TTCTTTCAGT AAATATTTAT TGAGTACCTA
301 CTGTGTGCTA GGCATTGACC TGGGAAGTAG AGATACTTCA CAGAATAACA
351 GGGAAAGTTC CCTGTGCTCA TGGAGCTTAC ATTCTACAGG GAGAAAGAGA
401 TAGCCAATAC ATAGGAATAA ATATATACAA GGTATCATGT AGTGATAATT
50 451 GCTGTGGAAA AAAAAAAGC TTAAGGGCGA ATTC

SEQ ID NO. 116: OC17-2-B,C

55
GAATTCGCC CTTGACCGCT
101 TGTTAAATAC CTTTGTCTAG CCTCTTCATA TGCTGTTGCA TATGACTCTC
151 ATCACAATC AGTGAGATGG AAAGACAAAT CCTATTTGTA CAAATGAGAA
201 AACTGAATC TTTAGAGTAA CTAGCTCAGT ATTGGCCAGC TGGTAAATGG
251 CAGTGTGGG ATTAATAATCC AGTCTCTATC TACTCTCCCT TTATTAGAA
301 GCATTATTG GATGTTGATC TTTGTTTCAG GTTTTGATTT TGTTACTTTT
351 TTATACTGTG TATATTTTCC TCAGTCTACC CTTCTGCTCT AGATTGTCTG
60 401 GACTCAGGAG ATTGTGGCAG TTAGTGATA GTTATTTTGA AGATAATGAT
451 TGCTTTTCTC TGTTTATATA AGTCATGTGT ACTTATTGTA GAAAGTTTGT
501 AAGATGCAAA AAGTATAAAA ATTAAAGTTA TGCATACTA ACATTTCAAT

551 ATATTTTCTC CCAGATTTTC AATAAAGACT TTCAGGCAGT GAAAAAAAAA
 601 AAGCTTAAGG GCGAATTC

SEQ ID NO. 117: OC17-3-A,B,C

5 GAATTCGCC CTTGACCGCT TGCACTGAA TTGGTTTGCA
 151 CACACTAACA TTTTACTCTA AAACAACATA GTTGCAATTGG AATCTGATGG
 201 AATATATTGA AACATATCCG TGACCTTTGA ATTGTAAGTA ATAAGTTGTG
 251 GAAAGTATAC TTAACCTGAC AGCATTAAAA ACAAATTAAT TTTGGTCTTA
 10 301 TCTTAAGATT TGAAGGCTA TATAAGGTAG TGACTGACCT ATGAAAGCTC
 351 TTTTATGTTG AAAGCAAGTG AAAAAAACT AAAGCCTTAT TGGTTTGAGG
 401 TTAGAACGGT TATTTGAAAA GTGGATTGA AAAGAAGTGA AGCTGAATTA
 451 TTCTAAAAAC AAAGGAATGA AGCTTTATGA CAGGGCACGT GAAATGTTTA
 501 TAGTGAAGAG GGAGAAATAA GTACCAATTG AAAAAAACTT CTAGAATTC
 15 551 ATTTAGTAAC AAAGAGGTTT TTGATGAAAA TTGTTTGGA AAAAAAAA
 601 GCTTAAGGGC GAATTC

SEQ ID NO. 118: OC17-7-A,E,J

GA ATTCGCCCTT GACCGCTTGT
 20 101 GAATAATATT GTCTCTATAG GTGTGCAAGC ATTCCTGGA AGCTATTGAA
 151 AACACAAGT ATGGCTGGTT TTGGGTATGC CCTGGAGGGG GTGATATTG
 201 CATGTATCGT CATGCACTTC CTCCTGGATT TGTGTTGAAA AAAGATAAAA
 251 AGAAAGAAGA GAAAGAAGAT GAAATTTTAT TAGAAGATCT AATTGAGAGA
 301 GAGCGTCTG CCCTAGGTCC AAATGTTACC AAAATCACTC TAGAATCTTT
 351 TCTTGCCTGG AAGAAAAGGA AAAGACAAGA AAAGATTGAT AACTTGAAC
 25 401 AAGATATGGA AAAAAAAA GCTTAAGGGC GAATTC

SEQ ID NO. 119: OC17-6-A,B,C

GAATTCG CCCTTGACCG
 30 101 CTTGTGTGGA GAAGGGGAAT AGAGGTGAAT TTAGGCTAAC CAGTTAGCTG
 151 AATGGTGGAC TCAGCCTTTG GCAGGAAAGA TTAAAGAGAA TTGGTTATTG
 201 GAAGTAGGGG TGATGAGGGC TTGTGACTGA TTGCATGTGG TGAGCAAAAG
 251 GGAAGAGGTG CCATGGGTGA TTTCCAGATT TTTGTTGGC AGGGGTAACT
 301 ACATTGGATG GATATGTCAA GATGGAGAAG GAGCAGATGA GGTAGGCATT
 35 351 CATTAATTTT TATTTAAATA TTTTCCTTTG TTGGGTATAC CTGGAGTGTC
 401 CTTTGTAGAG CCCCCGATTA GGCTCTGTCA GTGTGATAAA ACAAAATAGTT
 451 TTAAC TAGAA AAAAAAAG CTTAAGGGCG AATTC

SEQ ID NO. 120: OC19-4-A,C,G

GAATT
 40 101 CGCCCTTAAG CTTTTTTTTT TTCGTACTAC ACGACACGTA CTACGTTGTA
 151 GCCCACTTCC ACTATGTCCT ATCAATAGAA GCTGTATTG CATCATAGG
 201 AGGCTTCATT CACTGATTTT CCCTATTCTC AGGCTACACC CTAGACCAA
 251 CCTACGCCAA AATCCATTTC ACTATCATAT TCATCGGCGT AAATCTAACT
 301 TTCTTCCAC AACACTTTCT CGGCTATCC GGAATGCCCC GACGTTTGAA
 45 351 GGGCGAATTC

SEQ ID NO. 121: OC19-5-E

GAATTC
 50 101 GCCCTTAAGC TTTTTTTTTT TCGTACTACA CGACACGTAC TACGTTGTAG
 151 CCCACTTCCA CTATGTCCTA TCAATAGGAG CTGTATTGTC CATCATAGGA
 201 GGCTTCATT ACTGATTTC CCTATTCTCA GGCTACACCC TAGACCAAAC
 251 CTACGCCAAA ATCCATTTC CTATCATATT CATCGGCGTA AATCTAACT
 301 TCTTCCACA ACACTTTCT GGCCTATCC GAATGCCCCG ACGTTTGAG
 55 351 GGGCAATTC

SEQ ID NO. 122: OC19-5-F

GAATT
 60 101 CGCCCTTAAN CTTTTGTTTT TTAANANTGT NCTANNCTG NTTGTAAACN
 151 GCTATTTTAN AAAANANANC ATNCAGANNA CATGAANANC NCANAANNAT
 201 ATNAAAACAT CAAGNNCTTT TGTACATCAC TGTAGCATAA GCTGNTNGAG
 251 GTTGTNANGC AAAATACTAT NCTTCANGTG ACGGAAACA AGGNGGATGT
 301 TNTCCGTGTT GANAGCAGNG GTGAANNNGT GNNATANGG CTNATGTTGN

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351 TGGGCCCAAC NNTNGAAGGG CGAATTC

- 5 SEQ ID NO. 123: OC22-1-A,B 2F11
 GAATTCGCCCTTTGATCCCTGGATTATGCAAAGAAAAATGAACCCAAACATAGACTTGCAAGACATGGCCTGTAT
 GAGAAGAAAAAGACCTCAAGAAAGCAACGAAAGGAACGCAAGAACAGAATGAAGAAAGTCAGGGGACTGCAAAG
 GCCAATGTTGGTGCTGGCAAAAGTGAGCTGGAGATTGGATCACAGCCGAAGGAGTAAAGGTGCTGCAATGATGT
 TAGCTGTGGCCACTGTGGATTTTTTCGCAAGAACATTAATAAATAAACTTCATGTGAAAAAAGCTTAA
 GGGCGAATTC
- 10 SEQ ID NO. 124: OC22-1-C 2F12
 GAATTCGCCCTTTGATCCCTGGGACACATTCTCAAAAATAGTATTCCTTGGGCTTTATAGGAAGTCTGATGAGAG
 ACAATGTGGCTTTATTAGAGTGGAGAAGGTGCAGATGAAGAGATAACAGGCTCCAGGCATGTTTGGAGGCAATA
 GGCTAGATTTTAGGGGAGAAGTAACTAAGGAATTAGATAGTTTTTTCAGGTTTTAGCTTTGAACAATTGGGTGGT
 TGGTGGACACCGTTACTAACACTGGGAAACCTGAAAAAGAAAGATATTGGGGAAAAAAGCTTAAGGGCG
 AATTC
- 15 SEQ ID NO. 125: OC22-1-D
 GAATTCGGGCTTAAGCTTTTTTTTTTTCAGGTAGAATTTTTTCTACAAAAATGGTGATTTATTTAACATACAATGG
 TAGATATATTGGGGATCATCAAATTTTTAAAAATTTTTGTGGGCTTACTTTACATATTACAATACTAACGTAAG
 CTTATAAATCTTCCATTTTCAGTGAAGGAAAGGATCCAATTAACACCTGCTAGCAGCTCGGCAAGCCGAATTC

- 25 SEQ ID NO. 126: YA2-3-D,F
 GAATTCGCC CTTAAGCTTT TTTTTTTTAT TGTAATACT
 151 CTTTATTGTA AATATTCTAT CCTAAATTCC ATATAGCCAA TTAATCTTA
 201 CAGAATCTTT TGTTAATTTT TGTGTGTATA AATTTTACAG AGATAAAGGG
 251 TATGTTTGT GCACACAAT TACAAATAAT AATAAACTCT TTATTGTA
 301 TATTCCTTAT TGTAATTTCT TTATCCTAAA TTCCATATAG ACAATTGATT
 351 CTTACAGAAT ATTTTGTAA TATTTTTTTT TTTTGCCAAA CCTTGTATCC
 401 AAAATCAACT ATCACC CGA TTTGGCCATG ATTTGACAAA ATTAGACTAC
 451 AAATAAAAT CCTGATAAC GTACAGCTC GGCAAAGGGC GAATTC

- 35 SEQ ID NO. 127: YA2-3-E
 GAATTCGC CTTGCGCGAG
 101 CTGCTTTCCA AATAGGTATT GAATTTATGC ATTAGTCTGG TGATTTCACT
 151 TCTGTGAAAT ATTTTGGGAT CTATACCAAT TAAACATTTT CATAGTTCTG
 201 CCTATTGTCC TTCCCTGAGG CTCCATTGCT GCTTGGTGGC CATTCTCTGC
 251 CTTTTTACAG TCACCTGAAC AATGACCCAT CATCTCTTGC TTGCTTGAAA
 301 TCTTGCTGAA ATGTTCTCAT TTCCTGTTTG CTGTATGGGC TCGGGTGGGA
 351 TGTTTGTGG CTCTGTTGTG TTTATTCAAC AATTTGTACA TTATTTGTG
 401 TCCTTTACTA CTGTAAACAG TAAATATAG TTGGTAAAAA AAAAAGCTT
 451 AAGGGCGAAT TC

- 45 SEQ ID NO. 128: YA4-2-B,C,D
 AATCGG GCTGCATAAA TACACATTAT CTAATGTATT ATAATATTCA TAACAATCCT
 CTGTGTTATC TATAGCCCAT TTCACAGGTA AGAAAAAGA CTAAAAGACA
 TTTAAGTGAC TTGATTAATG AAAAAAATAG GCAGTCAACC TGAACATCAA
 251 CCCAAATATT CTTATTACAT CAATACAGCC TCTCAGCAAC CAATTAATC
 301 CAGAGAAATGA AAGAAGGGGA AGAAGGAATA AAGATTCTGA GTGAGGGAAA
 351 TAAATATGTA TAAGACGTGG CAGGTTTAAC TTTAAGGAGG TGGTATATTA
 401 CTTTGTCTA AAAAAAAGG GCTTAAGGGC GAATTC

- 55 SEQ ID NO. 129: YA9-2-A,B,C
 GAATTCGC
 101 CCTTGGGTAA CGCCATACTT CATAAGTGGT AAAGAAAGGT ATAAATTTG
 151 GAAACATTTT GTTGGGCATA GTAGTGATTG GGTGAAAAGG ATAAATTATA
 201 TCAAAATGAG AATGTGCTGT AATTGGAAGT AGGGAGCTAA AGGATGTTTC
 251 TTTCAGTTTA GTAGAACTGG AACGTTTTAC TATTAAACAT GGCTTTTATA
 301 AATGCATGGT CCAATAATT TATTCACTGT TAGTATTTAA TCACTGTCA
 351 GCTTATTAAT GTTTCTGTGA CCCATTAATG AATTTTAAAT TACAAAAAT
 401 TGTCTAGCAG CTACAGTTTA AAAATGAAAC TAGACATTAA AATAAATTTG
 451 ATAATTTTTT ATAAAAAAG AAAGCTTAAG GGCGAATTC

SEQ ID NO. 130: YA9-3-C,D

GAATTCG
 101 CCCTTAAGCT TTTTTTTTTT AATTTCACAA AAGTTTTCAC AAGGACAACG
 151 TTATAGAAGA AAACCCCCAG CAGTGGCTAG GTCATGCAGA ACCATTAATT
 201 GTCATACCTT GGCCCATCTT ATTCATCCTT GTTGCACTTT AGAGAGAGAA
 251 GTAAGCTATG TGAGTTTAC AATGCTTTTA AACTGTCATA TTTCTGTG
 301 AGCACTTAA CTGGCACAT CTTATAGTTA TAAATGTTCT GAGGGCGTTA
 351 CCCAAGGGCG AATTC

SEQ ID NO. 131: YA11-3-D,E

GAATT
 101 CGCCCTTAAG CTTTTTTTTT TTAGGCCAT TTGAGTATT TGTTCCTCAAT
 151 TAGGAGATA GTTGGTATTA GGATTAGGAT TGTTGTGAAG TATAGTACGG
 201 ATGCTACTTG TCCAATGATG GTAAAAGGGT AGCTTACTGG TTGCTCTCCG
 15 251 ATTCAGGTTA GAATGAGGAG GTCTGCGGCT AGGAGTCAAT TGGAGGACGG
 301 GCTTAGTGGG CGAAATATTA TGCTTTGTTG TTTGGATATA TGGAGGACGG
 351 CGATTGAAGG CGGAATTC

SEQ ID NO. 132: YA20-4-C,H

GAATT
 101 CGCCCTTAAG CTTTTTTTTT TTATAATGAT AATTTTATA CTTTTATTAC
 151 TTAGAAAATA ATTTATATTT TTCCATCATT TAAACAAAGA GTAGGCCTGA
 201 GTCTCATGCC TTTTGACACAG CTTTACCTT CAAAGAAAGT TATCTGGGTA
 25 251 AGATAGGCAG GAAATATGGG GAAACTGCAA ATTAACAGTC TACATACATC
 301 TAATATGAAC AGTCTGTAAG ATATTCCTTT TCTTTCGTTT TACTGGGATC
 351 GCAACAAGGG CGAATTC

SEQ ID NO. 133: YC1-5-F,H,I

GAATTCG CCCTTCAGGC
 101 CCTTCGATGT ATGCCATTTA GTGAAAGTGC TAAGTCTTAA GTTTCCTACC
 151 ACTTTGGTTT CATATTTTGT GACTTAACAA AGTTGTGAAT AGCACAGTCG
 201 AGGAAAATTG ATACCTGCAG TAACCCATAG GAAATAAACT GTAGAGTTCC
 251 ATATTCTGGT ATTGTGATTA TATTGTTTTA TATTAATAAG GAAAAGAAAA
 301 GAATTTTTTT TAATTTTATT TTTCCCGTC TTGCAAAAGTA TAGTGACCCC
 35 351 TGTTCCTATT AAATTTGAAT AAAGACTATT TTTGCTTGAA AAAAAAAAG
 401 CTTAAGGGCG AATTC

SEQ ID NO. 134: YC2-3-G,I,P,R,T 2E9

GAATTCG CCGCTTCCGCTGTGGGATCTGGCACTGTGGTTCTCTGCATGAAGACAGTGGCTGGCGGTGCCTGG
 40 ACGTACAATACCACTTCCGCTGTACAGGTAAAGTCCGCCATCAGAAGACTGAAGAAGTTGAAAGACCAGTAGACG
 CTCCTCTACTCTTTGAGACATCACTGGCCTATAATAATGGGTTAATTTATGTAACAAAATTGCCTTGGCTTGT
 AACTTTATTAGACATTCTGATGTTTGCATTGTGTAATACTGTTGATTGGAAAAGCATGCCGAGCTGGAAAAA
 AAAAAAGCTTAAGGGCGAATTC

SEQ ID NO. 135: YC4-2-B,C,D

GAATTCG
 101 CCCTTAAGCT TTTTTTTTTT CACGGAGGAT GGTGGTCAAG GGACCCCTAT
 151 CTGAGGGGGG TCATCCATGG GGACGAGAAG GGATTGACT GTAATGTGCT
 201 ATGTACGGTA AATGGCTTTA TGTACTATGT ACTGTTAAAG ATGGGTAGGT
 50 251 TTGTTGGTAT CCTAGTGGGT GAGGGGTGGC TTTGGAGTTG CAGTTGATGT
 301 GTGATAGTTG AGGGTTGATT GCTGTACTTG CTTGTAAGCA TGGGGAGGGG
 351 GTTTTGATGT GGATTGGGTT TTTATGTACT ACAGGTGGTC AAGTATTAT
 401 GGTACCGTGC AATATTCATG GTGGCTGACT AAGGGCGAAT TC

SEQ ID NO. 136: YC13-1-G,I

GAATTCG CCCTTAAGCT
 101 TTTTTTTTTT CGGTTAGGGT ACCGCGGGCCG TTAAACATGT GTCAGTGGGC
 151 AGGCGGTGCC TCTAATACTG GTGATGCTAG AGGTGATGTT TTTGGTAAAC
 201 AGGCGGGGTA AGATTTGCCG AGTTCCTTT ACTTTTTTTA ACCTTTCCTT
 55 251 ATGAGCATGC CTGTGTTGGG TTGACAGTGA GGGTAATAAT GACTTGTGG
 301 TTGATTGTAG ATATTGGGCT GTTAATTGTC AGTTCAGTGT TTTAATCTGA
 60 351 CGCAGGCTTA TGCGGAGGAG AATGTTTTCA TGTTACTTAT ACTAACATTA

Example 3***Age-Related Differential Gene Expression in Glioblastoma*****1. Patient Characteristics**

The 211 patients were diagnosed with glioblastoma multiforme (GBM) at the Chicago Institute for Neurosurgery and Neuroresearch between October 1, 1987 and December 30, 1994 and consisted of 94 females and 117 males. 180 patients had lesions confined to one cerebral hemisphere and 30 had lesions that were more extensive or were multifocal. All tumors were classified by the same neuropathologist essentially according to a four-tiered grading system typified by that of the WHO Classification scheme. Patients with high grade oligodendrogliomas, and mixed cell gliomas were excluded from the study. Survival was measured from the date of first surgery at CINN to the patient's death or the date of the last clinic visit, and updated to March, 1996. The strength of association between the survival times of different patient groups was determined using the modified Wilcoxon test.

2. Tissue Materials

For the Differential Display analysis, 3 GBMs excised from older (>60 yr.) patients, 3 GBMs excised from younger patients (<45 yr.) and 3 sections of normal gray matter were used. Their individual characteristics are listed below:

Normal	"Young" GBM (<45yr.; "Y")	"Old" GBM (>60 yr.; "O")
UMB 242 (46 yr., female)	CINN 319 (37 yr., male)	CINN 407 (64 yr., female)
UMB 418 (53 yr., male)	CINN 361 (40 yr., male)	CINN 422 (64 yr., male)
UMB 389 (71 yr., male)	CINN 504 (43 yr., female)	CINN 419 (72 yr., female)

Normal human brain tissue was obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, Maryland. Brain tumor tissue was obtained from the tumor bank maintained by CINN.

3. Differential Display

Total RNA from 3 individual specimens per patient group was extracted by guanidinium thiocyanate followed by cesium chloride sedimentation (Chirgwin, et al.) and treated with DNase I. Reverse transcription was performed utilizing single base anchored primers: (T11M, 5' TTTTTTTTTTMM 3', where M denotes A, C or G). Differential display was performed

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essentially as described (Liang, et al., 1992). For each of the three anchored primers in each sample, 28 arbitrary upstream primers were utilized in the PCR amplification to produce a total of 84 unique primer pairs in the analysis. The resultant amplicons were electrophoresed on 6% sequencing gels. Differentially expressed amplicons were excised, reamplified and purified.

5 They were subsequently subcloned into the TA cloning site of the pCR2.1 vector (Invitrogen, Carlsbad, CA) and insert-containing vectors from multiple positive transformants sequenced using an ABI 377 automated fluorescence-based sequencer. All NCBI maintained nucleotide databases (National Center for Biotechnology Information, Bethesda, MD) were searched for

10 homologies using the BLAST program (located at <http://www.ncbi.nlm.nih.gov/BLAST/index.html>).

4. Northern Blot

25 mg of total RNA isolated as above was electrophoresed through 1.2% formaldehyde-agarose gels and transferred to nylon membranes by capillary blotting. The membrane was

15 hybridized to a uniformly (³²P)-labeled hsp60 cDNA amplicon identified by differential display. This probe is homologous to the 3' end of the hsp60 protein coding region. Filters were hybridized for 90 minutes at 68°C using Express-Hyb (Clontech, Palo Alto, CA). Filters were washed in 0.1X SSPE/0.1% SDS at 50°C and analyzed by autoradiography for appropriate times.

20 CDNA probes for genes representative of the other major stress protein families, hsp27, hsp70, hsc72, hsp89 α , hsp89 β , and GRP78 were generously provided by Dr. Richard I. Morimoto.

5. Sequences identified as being over-expressed in "Old" tumors

Using the DDRT-PCR methodology described above, the following sequences were identified as being differentially expressed (ie, overexpressed in "old" tumors as compared to

25 "young" tumors) in tumor cells taken from patients older than 60 yrs, of age.

30 **SEQ ID NO. 142:** **OA 3-1-B**
 AGTCAGCCACCATGAACAAAGTGGATCTTGTCTTCTTACATCTATGAAAATAGAGCTTTGAA
 TGGTAAGGAGATATGTTTTCTTGGTAACCAATGCAAGATTGATGGGTGGAACATGATTCAA
 ACTTACACAATTTTTCTTGCTATTTTTCAAATATGAATCTTACTATATATTCTCGGTGAACA
 TCAGGAGACTATTAAAGAGGTCTGCTGTAAATGTAAAAAAGCTT

SEQ ID NO. 143: **OA 11-4-1**
 AAGCTTTTTTTTTTAGAAATCAGGNGKTTTTTTATTTAATACATTCTAATCAAATAGTAAC

AGCAGTAAATAAACACTTTGAAAAACAGGCAGGTATCCCCCTGTATCTGGAAGAAAATTAAG
TCAAAGTATTCTACACAGTAGAAGGGAGACAACTGTTTATGTCCATGGTTAGACAATTCAAG
GACAACTTGGATATTTCTAAAGCCATTTCCAAAAAATCAATGGCAACAGGTTGGGACACAGC
TATTTCAAAGGGTAGAATGCCTATACCTACATTGGTTTTTTATTAACGGCGATTGAAGCCGAA
5 TTC

SEQ ID NO. 144: OA 11-5-C

AAGCTTTTTTTTTTTAGCGACAGTTGTATTTATTTTTTTAAGTTACAATAAAATGCTCTCAA
10 GTCCCTTGAATGTTCCAACAAATTCAAACTTCATTTTCTGAATGTTTACATAAAATGCGAA
CTACCTGTTGCGATTGGNAACCTGCTGCTGTATTTTCATGTCTTAACGGCGATTG

SEQ ID NO. 145: OA 11-6-A

AAGCTTTTTTTTTTTACAAATGGAAGGTTTCTGACAACTTAAGTGGAGCAAGTACAAGTCT
15 ATCAGTGCAATTTTTCCAATAGCATATGCTTACTTCCATGTGTCATGTTTGGTAATTTTC
ACAAAATTTAACTTTATTACTATTATACCTGTTACGGCGATTG

SEQ ID NO. 146: OC 11-1C

CAATCGCCGTCATGGAGTGAATAATGAGTGAAAAAGTTTGATATTATCTATGTAATGAGT
20 TGATAACGACCTATTTTTTTTTAAAGAAGTCTTGCCTTTAAATAAAAACCTCAACTATAACAT
GTGGCACTTGATGTACATTCGCGTTCCATCTTCGTAAAAAGCCTGTGGAATAGGTAGGTATT
ATCTTTTATAGATGTGGAATGTAGGCTTCGTTATTTTAAAGCTTGTGGAAGCTTTACACA
GGTAGTAAGAGGCAGATTTGAACCTAGGCATTCTGATTGCAAGTAATTCCTTTTATTATG
CCACAGTGTGTTTATTATATACACTGAGTGTAGCTAATCGCCACTGGAGACGCCTTTGAAAA
25 AAAAAAAGCTT

SEQ ID NO. 147: OC 11-4-C

AAGCTTTTTTTTTTTTGAAGGAAAATTTGTATTATTTSAATTATTTTTATGKACAGAAAAC
30 CAACAGTGATACATTTAACCAGTTTAGKGGCAAGTTCTTTAGCCTTTGCCTTTTCGAGCTTG
GCGATACGAGCCACAGACTTAGGACCCAGGACACTGCCACCCCACTGACGGCGATTG

SEQ ID NO. 148: OC 12-3-3

GCTGATAGTGAATGAGTTCGAAAAAAGCTTAATATAGCAAGGACTAACCCTTA
TACCTTACTACCAGACAACCTTAGCCAAACATTTACCCAAATAAAGTATCGGCGATAGAAA
35 TTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATGAAAAATTATAACCAAGCAT
AATATAGCAAGGACTAACCCTTATACCTTCTGCATAATGAATTAAGTAACTAGAAATAACTTTGCA
AGGAGAGCCAAAGCTAAGACCCCGAAACAGACGAGCTACCTAAGAACAGCTAAAAGAGCA
CACCCGTCTATGAAAAAAGCTT

SEQ ID NO. 149: OC 12-4-1

GGCTTAAGCTTTTTTTTTTCAAAAATACAAAATAAATTATTTGTAGGCATGGACAATGACAG
40 CAGTAACTGNTATTTATTTGTCAGCTGAAATCAGTAACTGATGGTTGTAGTGATTTTTTAAA
AACATCACCCAGCATTTTCTCAGTCATTTTCTTCAAATGACTTCTCTGTAGTTACTGGAGA
GAAATACTGCCTTGAGCTTCTATCGCCGA

SEQ ID NO. 150: OG 14-4A

TCTGTGCTGGGAACTGGCTAACTGTATGCAAAAAACAGAACTGGACCCCTTCTTACACC
45 TTATACAAAATTAAGTCAAGATGGATTAATTAAGACTTAAACGTAAACCCAAAACCATAA
AAACCCTAGAAGAAACCTGGGCAATACCATTACGACATATACTTGGCAAGGTCC

SEQ ID NO. 160: OC 15-2-C

TAAGCTTTTTTTTTTTCGGGTGTGCTCTTTTAGCTGTTCTTAGGTAGCTCGTCTGGTTTCGG
50

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GGGTCTTAGCTTTGGCTCTCCTTGCAAAGTTATTTCTAGTTAATTCATTATGCAGAAGGTAT
AGGGGTTAGTCCTTGCTATATTATGCTTGGTTATAATTTTTTCATCTTTCCCTTGCGGTACTA
TATCTATTGCGCCAGGTTTCAATTTCTATCGCCTATACTTTATTTGGGTAAATGGTTTGGCT
AAGGTTGTCTGGTAGTAAGGTGGAGTGGGTTCGGAA

5 **SEQ ID NO. 161:** OA 16-5-II
AGCCAGCGAAGAAGAAAGGGGAACCAACAACCTCAAATGTGGGCAACCAGCATCTGTCTCAG
GGAAGGAAAGCATGTGAGAGAATTTCTGGTTAATGATTGGGGGTAGAAAAGGCCATTGGAAA
ATAGAACCCCTGGATCCTTTTGGAAAGGTGAGGGTTGGGGTTCTGGGCCTTCTATGTCTCTT
10 CTGTATCTAAAAAAAAGCTT

SEQ ID NO. 162: OC 16-4-A
TAGCCAGCGAAGATAGAAAGGTAGTCCCTGGTCAGTCATTAKTATTGGTAAGAGTTAAAATT
AGCAATATATTTAAATTTCTTTCAATTCATGTACGAGTCTTCCCCCAGCCCTTCACTGGGTG
15 ATACATGTAAGGATTAGGYGTTAGKGAGACAGCTGTAGTCGYACTCAMCATCTGARCCAAGW
AGATAGTCATCATTTTTCTTCTCTTGATTYACTTGAAAAAAAAGCTT

SEQ ID NO. 163: OC 16-7-A
AAGCTTTTTTTTTTTCAGATGWGWTCAATTTATTATGCTTTTTAAACTTARGTACATGKTAC
20 ATATATTCATTTTAAATGCCTTGATACAAATAAAAAAGGAAAGCACATATATACAAATAAGA
ATGCCACTATCATGGGATAACTTTGAACCTGCTTAAAGTTTCTCAATTAACGTATTCACAA
GCTTCAGTACTGTAACATATTCGCTGGCT

SEQ ID NO. 164: OA 17-4-D
25 GACCGCTTGTTAAGAGGAAGTATCTCATATATTTGTATCAGAACTGTATTTTTATGTTATA
TTGTATAGTTTGCTCTCCTGCCCTCTCCTTAAACTGAATGGTGCCAATAATTTGATACTA
ATGACTACAAAAAAGGTAATGCCTCATTTACTAGTATTGTTGTAATGAGGAATGTATGT
GAATATTCAGATAACCGAGGATTAACCCCTTAAAGTGTGAATCTTTAAATTTTAAATATAT
TTTTTTTGAGGGAATCTTTCTAAATGTATTACGCACTCCCTGCCTTAGTAAACAGAGTA
30 TACTGGAGAGTATTTAACCTTTTCTTGATGAGTCATGGCATGATTATAAACATCAGCCCTT
TTAAAAAAAAGCTT

SEQ ID NO. 165: OC 17-5-E
35 GACCGCTTGTTGATGGAGAAGGGGAGAGCATCTAGGCAGGCAAACAGAAGGGAAGTGGAGTT
AAACCTCTGGCATGAAGTCTGGGAGTAGGGTAGGCTAGGGGGTTTCTTCTATGACACTTGAC
CCTTCCATGCTGGTCCCAAGCCTATTGGAGGAATGTGGGTGTGGCCGAGGTGATGGCAAGA
AAGGTGCAAGAAAGTGAGCAGTCTGCCTGTGAGTGAGCACAGATGCCGGGGTGTGTGTGTGT
GTGTGTGATTTTCACTGTGGGGTGTGTCTGTGAGAGCTAGCTGCCTTACCCCTCCTTGGCAC
ATAGTAGGCCTTCCATAATGTTGGATGGATGGATAAATAGATTGGGACCATCAGACCATGA
40 AAAAAAAAAGCTT

SEQ ID NO. 166: OC 17-8-A
AAGCTTTTTTTTTTCCAGAAAAAACAACATGCAACACTTCGATTTTCAACTTCCAGCAC
CCAAACTGTGAGAAAATAAATGTCTGTCTGTGTAAGCCAACCAAGTTGTGGCATTTTCTTAT
45 GGCAGCCCTAGAAAAATAACATACAGTTTTCCTCCTATATCTACCTGTCAGTAATGAGAAG
TTCAAAAGGACACTAGGCTATTGCTTATTAAGAAAGAAACAACACAAAAAACAACCTCT
TTCTTGACTTAGGATATTTAAGAAGATTATGCAGAACACTTAATTTCTCCCTATTTTCCT
TATACAAGCGGTC

SEQ ID NO. 167: OC 17-12-A
50 AAGCTTTTTTTTTTCTAAAAATTGCAAAAAGGGACGCCACATTGGKGACAGAAAGCCTGGTT

5 TCACTTCACGGAATAAGCAGTTTGAGATCAATGTCCCAGAAGAGTTTTGACATTCAGGACTT
AAAATAGCAGCAGCAGCAGAGGTAGCTGAAATGGCAAGTAATGAAAATTGCTTTAGTAA
AAATATTTTGGACTGAAGGTATGAGAACTAAAAGTAGAACTAGTAAGACACAAAGCATAA
CATGACCAGGAATCTGATACAGTAGTGAACAAGCGGTC

SEQ ID NO. 168: OC 17-12-B

10 AAGCTTTTTTTTTTCTGATTAAGTTACAAACATTCTCCCTATAGCTAAACTCCGTGACTAG
GCTCCCAGCCTCATGGCCAAGAACAATAAGTTCACCCACTTATCTGGAGTAACCATAGTA
TTAAAGAAATACAATTCTTTCTTCTAAAGACAATTTCCAGAAAGACCTGCCTTTCCCTATGG
GTACTTGACACTAGGTCCCAGCACAGGCTAATCGCTGTATGGTTTCTTGAAGATTGGCTTT
TCTEAGTTTCTTTCTTTTGATACTGTACAAGCGGTC

SEQ ID NO. 169: OC 17-12-D

15 AAGCTTTTTTTTTTCTAGAGTGTTATTGCTCCATCACCTAGGCTTGAGTGCAGKGGTGTG
ATCTTGGCTCACTGCAGCCTCAACCTCCTGGGCCCCAAGCAATCCTCCACCTCAGCCTCTTG
AGTAGCTGGGACCACAGACGTGCACCACGAGACCCAGCTAATTTTTAATTTTTTTGTAGA
GGTGGGGGTCTTCCTATGTTGCCAAGCTGGTCTCAGACTCCTGAGTTCAAGTGATTCTCCC
ACCTAAGCCTCCCAATGTTCTGAGATTACAAGCGGTC

20 SEQ ID NO. 170: OA 19-5-2

25 AAGCTTTTTTTTTTAAAGATTGTTCTAATTCTGGTTGTAACTGCTATTTTAAAAACAAAA
CAAACAGAAAACATCAAAAACACAAAAGATATTAACAGCAAGTCTTTGTACATCACTG
TAGCATAAGCTGCTTGAGGTTGTATGCAGAAATAGTATCCTTCACGTACGGAAAACAAGGC
GGATGTTCTCCGTGTTGATAGCAGTGTTGAAGTGGTGGTATAAGGGCTTCTGTTGCTGGTCC
CGACGTTTGAAGC

SEQ ID NO. 171: OC 19-1-1

30 GCTTCAAACGTCGGATGGGAATTATGTCACCAAACAGGAGCTCAAAGGATTAGATATAGTTA
GAAGAGATTGGTGTGATCTTGCTAAAGACACTGGAAACTTTGTGATTGGCCAGATTCTTTCT
GATATAAACACCAATAGCACCAATCTGGAAGAAGTATTTAAGTTGGGAAACAAGGTAAAAAG
TGAAGTGAATAAGTTGTACAACTGCTTGAAATAGACATTGATGGGGTTTTCAAGTCTCTGC
TACTGCTGAAAAAAAAGCTTAAGC

SEQ ID NO. 172: OC 19-2-1

35 GCTTAAGCTTTTTTTTTTTCGCAAACTCATCACTAGACATCGTACTACACGACACGTACTAC
GTTGTAGCCCACTTCCACTATGTCCTATCAATAGGAGCTGTATTTGCCATCATAGGAGGCTT
CATTCACTGATTTCCCCTATTCTCAGGCTACACCCTAGACCAAACCTACGCCAAAATCCATT
TCACTATCATATTCATCGGCGTAAATCTAACTTTCTTCCCAACACTTTCTCGGCCTATCC
GGAATGCCCCGACGTTTGAAGCC

40 SEQ ID NO. 173: OA 21-2-2

45 GGCTTAAGCTTTTTTTTTTAAAGATTGGGNCTAATTCTGGTTGTAACTGCTATTTTAAAAA
ACAAAACAAACAGAAAACATCAAAAACACAAAAGATATTAACAGCAAGTCTTTTGTACA
TCACTGTAGCATAAGCTGCTTGAGGTTGTATGCAGAAATAGTATCCTTCACGTACGGAAAAA
CAAGGCGGATGTTCTCCGTGTTGATAGCAGTGGTGAAGTGGTGGTATAAGGGCTTCTGTTGC
TGGTCCCGACGTTTGAAGC

SEQ ID NO. 174: OC 24-1-E

50 AAGCTTTTTTTTTTCAAGGSTAATCAACAAGCTGAGGGAGTGAAAAAGAACAAGAAATC
TGTGACTGCTTGTGATCAATTAGTAACTTAATTTTTTAGATTAAATGAAATAATACATGC
AAAGCCCTTGGCACAGTGCCTTGCACATAATACATTTCCGGGGTTAAGTTGYGCTAGCTATTC

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TGTTATTGATTGNCTTGCCCTTTGTTCTCTGGAAGGTTGGATCTTGCCATTTGGGGATGGCC
 AATGGGAAGGCTGAGCAAGACATCAGAGGGTGGGAGGAAAGGAGGTATATTTATTTCCCTTA
 CTCCTTCTCTGCTGGGCTTCAATTTGTCACTAGCTGCATTCCTTTTATGACCACMACTCC
 MGTCC

- 5 Among the above-described sequences, Heat Shock Protein 60 (HSP60; SEQ ID NO. 149) was further characterized (Figure 2, Figure 3, Figure 4). HSP60, produced primarily in response to pathophysiological stress, is localized to the mitochondrial matrix and facilitates protein folding, translocation and assembly. Northern analyses revealed that the constitutive expression of HSP60 in normal brain is attenuated with increasing age. In stark contrast, HSP60 demonstrated robust expression in GBMs from older patients, inversely correlating with survival. A similar relationship between patient prognosis and the expression of most other major stress-inducible proteins was not observed. Taken together, these results suggest that this selective increase in HSP60 expression is not part of a generalized stress response and that modulation.

15

6. Sequences identified as being over-expressed in "Young" samples

- Using the above-described Using the DDRT-PCR methodology described above, the following sequences were identified as being differentially expressed (ie, over-expressed in "young" tumors as compared to "old" tumors), the following sequences were identified as being differentially expressed in tumor cells taken from patients younger than 45 yrs, of age.

25 **SEQ ID NO. 175:** NC 11-3-B
 GGCTTAAGCTTTTTTTTTTTCGCAAAATCAGGACAATTCGACAGTCTTTCCCCACTCCTTTC
 CCCAAATAGGAACGTAATCTCATATTAAAGGAGAAGCTGAACAAAATGGAATAGATGACTTG
 AGAAGGAGAAARAGGAGAAAGGAGACCATTACGACTGAGAGAAAATAGTTAATTTTAAGTGAC
 ATTTGTGGCACAGGAAGATTGAGAGTTTCATAGKACAAAGAAAGAGGTATCAGAAAAAAGTT
 TCCTACCATTACGGYGATTGAAGC

30 **SEQ ID NO. 176:** NA 12-2-A
 AAGCTTTTTTTTTTTTACATAGACGGGTGTGCTCTTTTAGCTGTTCTTAGGTAGCTCGTCTGG
 TTTTCGGGGGTCTTAGCTTTGGCTCTCCTTGCAAAGTTATTTCTAGTTAATTCATTATGCASA
 AGGTATAGGGGTAGTCCTTGCTATATTATGCTTGGTTATAATTTTCATCTTTCCCTTGCG
 GNACTATATCTATTGCGCCAGGTTTCAATTTCTATCGCCG

35 **SEQ ID NO. 177:** NC 15-1-1
 AAGCTTTTTTTTTTTTTCAGAATANGGGAATATATTTTTTAAGACAACCTNTTGTGGAAAAGT
 TCTGGGACAGTTTTCTCCAAGTGGCTTCTACCCTAAAGTCCCTCTAGCAAAATTTTAGGGTC
 TCCACACTCACGACAGATGTCCAGTCCCAAGACATATATCATNTTTTGGCACTTCCCCAAC
 CCCTCTCCAACACGTTCTGAATTAGATTACCCCAATAACTTTGATTCTGCGTGTAGATGT
 40 TTCTTCAGGCTATCCTGCCCTGGTTGGTGGGTTCCGA

SEQ ID NO. 178:**NA 15-3-B**

AAGCTTTTTTTTTTTAGAGGGTCTGTGGGCAAATTTAAAGTTGAACTAAGATTCTATCTTG
 GACAACCAGCTATCACCAGGCTCGATAGGTTTGTGCGCTCTACCTATAAATCTTCCCACTAT
 TTTGCTACATAGACGGGTGTGCTCTTTTAGCTGTTCTTAGGTAGCTCGTCTGGTTTCGGGGG
 5 TCTTAGCTTTGGCTCTCCTTGCAAAGTTATTTCTAGTTAATTCATTATGCAGAAGGTATAGG
 GGTTAGTCCTTGCTATATTATGCTTGGTTATAATTTTCATCTTTCCCTTGGGTTACTATAT
 CTATTGCGCCAGGTTCAATTTCTATCGCTATACTTTATTTGGGTAAATGGTTTGGCTAAGG
 TTGCTCTGGTAGTAAGNGGAGTGGGTTTCGGAA

10 SEQ ID NO. 179:**NC 21-1-2**

GGCTTGTTCGCTCCAAAGGGTGTATTAATTTCTGAATGCTAATCATGAAGACTTTRGTTAGG
 ACAACACTTCAAACCAGGAAGTGTGAAGTATTAGATTATAGCTACACAATTTCTGTGTG
 TTAGATCATGGGGTAGTTTGTAGTGTTCACATGTATTGCTATAAAATCACAGTGTACCAAG
 15 CTCTGGTTTAAATATGCCATTAATACTAATTAATAGAGCTGCTAGTCTCTCTGGAACAAAAA
 AAAAGCTT

SEQ ID NO. 180:**NC 21-1-4R**

CAATCGCCGTAATGGTAGGAACTTTTTCTGATACCTCTTTCTTTGKRCTATGAACTCTC
 AATCTTCCTGTGCCACAAATGTCACTTAAATTAACATTTTCTCTCAGTCGTAATGGTCTC
 20 CTTTCTCCTCTTCTCCTTCTCAAGTCATCTATCCATTTTGTTCAGCTTCTCCTTAATATG
 AGATTACGTTCTATTTGGGGAAAGGAGTGGGGAAAGACTGTGCAATTGTCTGATTTTGGC
 AAAAAAAAAAAGCTT

SEQ ID NO. 181:**YA 30-2-1**

25 CTGCTGGGACTATGGTACTAAATCCRGNAGATGGGCTGTGTAGCAACTCTCCAGGGGAACAC
 ACTAGGGTACTTAGGGAGGTGCTTTGTGGAGCATGTTGAAGCTTTGAGATCTGAGCAGGAGG
 CAGTGATGTCCCTGGTCTATTCAGGGAAAGATTTTCAAGTGTGAAATGGTAAACATCCAATTGA
 CAGGATTTAGATTTTGTCTAGTTTTCTGCTTTTTTAATGTTTCTATCCCCATCTCAGTGTT
 30 TTCTTTATCCATCCCAGTGATGCCTTATTTGAACTGGGCTTAACTGCAAAAAGAATGAAG
 TTGGATTTAGGAAGCTGTAGATCATTGAGTGGNGNTGAGAGTGAAGTTCCTAGCAGGGAA
 GTTTCCTTGAGCCTAAATAAAAAAGAAAAATTAATAAAGAATCMYGTTTTTTTAATTWAAAA
 AAAAAAGCTT

SEQ ID NO. 182:**OA16-4-A**

35 AAGCTTTTTTTTTTAAAGATAAATGTTGAATTGCAGGAAGAATAACATTTTGGAACAGTAAT
 GTGGGATATAAGAAAAAGTCACATAGCTCCAAATTTAGGGTGAGACTTTACATGTCTTAGAA
 GACCATTAAGAGGACTTCCAACAAGTAGGGGAGACCAAGTTTCAATTAGGGCAGAAGATAGG
 GAAGGAAGTCTATAAAGAGACTAAACTGTGAGGGTTCGCTGGCT

40 It should be noted that the sequence "GAATTC" at the 5' or 3' ends of the sequences may
 represent a restriction enzyme site used in characterizing the sequences and does not necessarily
 constitute part of the differentially expressed sequence.

Table 1**AGE-DEPENDENT GENES ASSOCIATED WITH GLIOMA PATIENT SURVIVAL BY DDRT-PCR ANALYSIS****(Normal⁺, Young⁺, Old⁻)**
NOVEL (6)

- 5 **Normal⁻, Young⁻, Old⁺**
- known ESTS (5)
 - STAT-induced STAT inhibitor-2
 - Fibrillin-15
 - NPA6, cri-du-chat
 - 10 • Ribosomal Protein L7a
 - Mitochondrial sequences (3)
 - Chaperonin (HSP60)
 - Glypican 3 (GPC3)
 - CDC42
 - 15 • Glucosamine-6-Phosphate Deaminase
 - Oscillin
 - Eph-like Receptor Tyrosine Kinase
 - SHOX-b
 - Cyclophilin-like Protein, CyP-60
 - 20 • KIAA0570
 - Guanine Nucleotide Binding Protein
 - DNA Polymerase (-subunit)
 - NOVEL (8)

Example 5

25

Reverse Northern Screening of RNA

- A 4 μ l aliquot of the purified cDNA amplicons is then reamplified, using similar conditions as described above, without radioactive isotope, and in the presence of 20 μ M dNTP. Following electrophoresis through 1.5-2.0% agarose, the amplicons are purified using
- 30 QIAquick^(R) gel extraction (Qiagen, Inc., Valencia, CA) and reconstituted in a total volume of 40 μ l. Duplicate 4 μ l aliquots of this gel purified cDNA are reamplified and combined in a total volume of 150 μ l for reverse Northern analysis. To this sample, 6 μ l of 10N NaOH is added, and the mixture incubated at 4°C for 10 minutes to denature the nucleic acids. The mixture is then diluted 1:1 with 150 μ l of 2 M NH₄OAc, 150 μ l of which is applied to duplicate nylon
- 35 membranes presoaked with 1 M NH₄OAc. Wells of the slot-blot apparatus (Schleicher & Schuell, Keene, NY) are washed with 150 μ l of 1 M NH₄OAc and filters rinsed in 6X SSC and soaked for 15 minutes in 2X Denhardt's solution, and air dried. The filters are UV-crosslinked in a Stratlinker apparatus (Stratagene, LaJolla, CA), and prehybridized for 2-4 hours at 57°C in 10%

dextran sulfate, 1 M NaCl, 1% SDS, and 50 ug/ml sheared salmon sperm DNA. The radiolabeled probe is prepared by reverse transcription (RT) of 10 ug total RNA from normal fetal astrocytes, or glioma cell line U373MG cells, utilizing the above conditions. Following RT, probe is treated with 20 ug RNase A for 30 minutes at 37°C and purified by Sephadex G50 chromatography. Equivalent amounts of radiolabeled probe ($2-3 \times 10^6$ cpm/ml) are added to the respective blots and hybridized overnight at 57°C. Blots were washed in 2X SSC/1% SDS at 57°C for 30 minutes and autoradiographed for an appropriate time.

The minimal selection criteria for the bands of interest is approximately two-fold greater signal expressed in either tissue, and is qualitatively evaluated by visual inspection of the autoradiographic image.

The amplicons determined to be differentially expressed (either glioblastoma or normal brain tissue specific) are subsequently subcloned into the TA cloning site of the pCR(R)2.1 vector (Invitrogen, Carlsbad, CA) and insert-containing vectors from multiple positive transformants sequenced using an ABI 377 automated fluorescence-based nucleic acid sequencer. All NCBI maintained nucleotide databases (National Center for Biotechnology Information; Bethesda, MD) are searched for homologies using the BLAST (basic local alignment search tool) program. As should be noted by the skilled artisan, use of the TA cloning site occasionally results in the inclusion of a poly-A or poly-T sequence at the 5'- or 3' end, respectively, of the cloned insert. Such sequences are not required to perform the assays described herein.

Following this procedure, the following sequences were found to be overexpressed in tumors of "old" patients as compared to "young" patients. The primers utilized to amplify each of the amplicons is described as well. The sequences are:

QA 3-1-B (SEQ ID NO. 142) NOVEL

GAATTCGCCCTTAGTCAGCCACCATGAACAAAGTGGATCTTGCTTCTTACATCTATGAAAATAGAGCTTTGAAT
GGTAAGGAGATATGTTTTCTTGGTAACCAATGCAAGATTGATGGGTGGAACATGATTCAAACCTTACACAATTTT
TCTTGCTATTTTTCAAATATGAATCTTACTATATATTCTCGGTGAACATCAGGAGACTATTAAAGAGGTCTGCTG
TTAAATGTAAAAAAGCTTAAGGCGAATTC
OLIGO
LEFT PRIMER start len tm gc% any 3' seq
19 20 58.26 45.00 6.00 2.00
CCACCATGAACAAAGTGGAT
RIGHT PRIMER 205 21 59.15 47.62 5.00 3.00
CTCCTGATGTTTACCGAGAAT
SEQUENCE SIZE: 260
PRODUCT SIZE: 187

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OA 11-4-1 (SEQ ID NO. 143) STAT-induced STAT inhibitor (STATI2)

GAATTCGGCTTAAGCTTTTTTTTTTAGAAATCAGGNGKTTTTTTTATTTAATACATTCTAATCAAATAGTAACAG
 CAGTAAATAAACACTTTGAAAAACAGGCAGGTATCCCCCTGTATCTGGAAGAAAATTAAGTCAAAGTATTCTACA
 CAGTAGAAGGGAGACAACTGTTTATGTCCATGGTTAGACAATTCAAGGACAACCTGGATATTTCTAAAGCCATT
 5 CCAAAAAATCAATGGCAACAGGTTGGGACACAGCTATTTCAAAGGGTAGAATGCCTATACCTACATTGTTTTTA
 TTAACGGCGATTGAAGCCGAATTC
 OLIGO $\frac{\text{start}}{100} \frac{\text{len}}{20} \frac{\text{tm}}{59.67} \frac{\text{gc\%}}{55.00} \frac{\text{any}}{4.00} \frac{3' \text{ seq}}{2.00}$
 LEFT PRIMER
 AGGCAGGTATCCCCCTGTAT
 10 RIGHT PRIMER $\frac{266}{21} \frac{59.99}{47.62} \frac{6.00}{1.00}$
 TGAATAGCTGTGTCCAACC
 SEQUENCE SIZE: 324
 PRODUCT SIZE: 167

15 OA11-5-B/C (SEQ ID NO. 144) NOVEL (fibrillin homology)

GAATTCGCCCTTAAGCTTTTTTTTTTAGCGACAGTTGTATTATTTTTTTAAGTTACAATA
 AAATGCTCTCAAGTCCTTTGAATGTTCCAACAAATTCAAAACCTTCATTTCTGAATGTTTTA
 CATAATGCGAACTACCTGTTTCGATTGGNAACCTGCTGCTGTATTTCATGCTTAACGGCG
 20 ATTGAAGGGCGAATTC
 OLIGO $\frac{\text{start}}{17} \frac{\text{len}}{22} \frac{\text{tm}}{60.02} \frac{\text{gc\%}}{40.91} \frac{\text{any}}{4.00} \frac{3' \text{ seq}}{3.00}$
 LEFT PRIMER
 CGCCGTTAAGACATGAAATACA
 RIGHT PRIMER $\frac{137}{21} \frac{58.09}{42.86} \frac{4.00}{3.00}$
 TGCTCTCAAGTCCTTTGAATG
 25 SEQUENCE SIZE: 202
 PRODUCT SIZE: 121

30 OC 11-4-C (SEQ ID NO. 147) RIBOSOMAL PROTEIN L7a

GAATTCGCCCTTAAGCTTTTTTTTTTCGAAGGAAAATTTGTATTATTTSAATTATTTTTATGKACAGAAAACCT
 AACAGTGTACATTTAACCCAGTTTAGKGGCAAGTTCTTTAGCCTTTGCTTTTCGAGCTTGGCGATACGAGCCAC
 AGACTTAGACCCAGGACACTGCCACCCAGTGACGGCGATTGAAGGGCGAATTC
 OLIGO $\frac{\text{start}}{68} \frac{\text{len}}{26} \frac{\text{tm}}{57.47} \frac{\text{gc\%}}{34.62} \frac{\text{any}}{6.00} \frac{3' \text{ seq}}{2.00}$
 LEFT PRIMER
 35 GAAACTCAACAGTGTACATTTAACC
 RIGHT PRIMER $\frac{173}{20} \frac{59.72}{60.00} \frac{3.00}{3.00}$
 GCAGTGTCTGGGTCCTAAG
 SEQUENCE SIZE: 205
 PRODUCT SIZE: 106

40 OC 12-4-1 (SEQ ID NO. 149) HSP 60

GAATTCGGCTTAAGCTTTTTTTTTTCAAAAATACAAAATATTTGTAGGCATGGACAATGACAGCAGTAAA
 CTGNTATTTATTGTACGTGAAATCAGTAACCTGATGGTTGTAGTATTTTTTAAAAACATCACCAGCATTCTCT
 TCAGTCATTTTCTTCAATGACTTCTCTGTAGTTACTGGAGAGAAATACTGCCTTGAGCTTCTATCGCCGAAAG
 45 CCGAATTC
 OLIGO $\frac{\text{start}}{50} \frac{\text{len}}{20} \frac{\text{tm}}{59.68} \frac{\text{gc\%}}{50.00} \frac{\text{any}}{5.00} \frac{3' \text{ seq}}{2.00}$
 LEFT PRIMER
 TAGGCATGGACAATGACAGC
 RIGHT PRIMER $\frac{209}{22} \frac{59.51}{50.00} \frac{3.00}{1.00}$
 50 GCTCAAGGCAGTATTTCTCTCC
 SEQUENCE SIZE: 233
 PRODUCT SIZE: 160

55 OC 16-4-A (SEQ ID NO. 162) NOVEL

GAATTCGCCCTTAGCCAGCGAAGATAGAAAGGTAGTCCCTGGTCAGTCATTAKTATTGGTAAGAGTTAAATTAG
 CAATATATTTAAATTCTTTTCATTTTCATGTACGAGTCTCCCCAGCCCTTCACTGGGTGATACATGTAAGGATT
 AGGYGTTAGKGAGACAGCTGTAGTCGYACTCAMCATCTGARCCAAGWAGATAGTCATCATTTTCTTCTCTGA
 TTYACTTGAAAAAAAAGCTTARGGGCGAATTC
 OLIGO $\frac{\text{start}}{\text{len}} \frac{\text{tm}}{\text{gc\%}} \frac{\text{any}}{3' \text{ seq}}$

LEFT PRIMER 28 22 60.03 50.00 5.00 2.00
 AAAGGTAGTCCCTGGTCAGTCA
 RIGHT PRIMER 143 21 57.37 47.62 6.00 0.00
 ACATGTATCACCAGTGAAGG
 SEQUENCE SIZE: 260
 PRODUCT SIZE: 116

OA 21-2-2 (SEQ ID NO. 173)**Guanine Nucleotide Binding Protein α 13 (GNA13)**

10 GAATTCGGCTTAAGCTTTTTTTTAAAGATTGGGNCCTAATTCTGGTTGTAACTGCTATTTAAAAACAAAAAC
 AAACAGAAAAACATCAAAAACAAAAAGATATTAAACAGCAAGTCTTTGTACATCACTGTAGCATAAGCTGCT
 TGAGGTTGTCATGCAGAAATAGTATCCTTCACGTCACGGAAAAACAAGGCGGATGTTCTCCGTGTTGATAGCAGTGG
 TGAAGTGGTGGTATAAGGGCTTCTGTTGCTGGTCCCGACGTTGAAGCCGAATTC
 OLIGO
 15 LEFT PRIMER start len tm gc% any 3' seq
 TGCTTGAGGTTGTCATGCAG 147 20 61.02 50.00 5.00 2.00
 RIGHT PRIMER 258 20 59.88 50.00 4.00 2.00
 ACCAGCAACAGAAGCCCTTA
 SEQUENCE SIZE: 280
 PRODUCT SIZE: 112

OA7-1-B (SEQ ID NO. 184) Guanine Nucleotide Binding Protein α 13 (GNA13)

25 GAATTCGCCCTTCAAACGTCGGGACCAGCAACAGAAGCCCTTATACCACCACTTCACCACTGCTATCAACACGGA
 GAACATCCGCCTTGTTTCCGTGACGTGAAGGATACTATTCTGCATGACAACCTCAAGCAGCTTATGCTACAGTG
 ATGTACAAAGACTTGCTGTTTAAATATCTTTTGTGTTTTGATGTTTCTGTTTGTGTTTTTAAATAG
 CAGTTTACAACAGAATTAGAACAATCTTAAAAAAAAGCTTAAGGGCGAATTC
 OLIGO
 30 LEFT PRIMER start len tm gc% any 3' seq
 ACCAGCAACAGAAGCCCTTA 24 20 59.88 50.00 4.00 2.00
 RIGHT PRIMER 250 26 60.00 34.62 4.00 2.00
 TTGTTCTAATTCTGGTTGTAACTGC
 SEQUENCE SIZE: 281
 PRODUCT SIZE: 227

The following sequences were determined by DDRT-PCR and reverse northern assay
 35 to be over-expressed in cancer cells, regardless of the age of the patient from whom the tumor
 sample is isolated. The sequences and primers utilized to amplify the sequences are shown
 below:

NC17-10-A.H**STM 2 (SEQ ID NO. 68)**

40 GAATTCGCCCTTGACCGCTTGTAAGGGAACAGAGACAGAATGAAATGAAAGAAGGCAG
 TTGAACCTTCTAGGCTTCTACAGGCAGAAAAACAGGCTGATAGAACTGCTCAACTACAGACATG
 TTCTACCTTTCTAGAAAAAAAAGCTTAAGGGCGAATTC
 OLIGO
 45 LEFT PRIMER start len tm gc% any 3' seq
 GCTTGTAAGGGAACAGAGA 17 23 59.94 47.83 4.00 0.00
 RIGHT PRIMER 131 25 59.77 44.00 6.00 2.00
 GGTAGAATGTCTGTAGTTGAGCA
 SEQUENCE SIZE: 165
 PRODUCT SIZE: 115

NC17-10-B,C,D**NOVEL (SEQ ID NO. 69)**

GAATTCGCCCTTGACCGCTTGTTGACAGGATATGGGAGATGGAAAAGGAAAGGATCTGCATC
 TAGTGATTGGAAATATAGGAGTGGTGGGGTTAGTTTCAGATGCCTGTGGGATATTAATGT
 CCTGTGTTGAGTTGGAATATGAGTTCTACAGAGGGCAAGATTTAGGAGTTGGCACTCCTAA

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GTGTCAATACATGTGAATAGGATCGCTTTGGAGGGTGAGAAGAGGTCTGAGAACACTACTAG
 GGAACAGTGAAGGAAAAAAAAAAGCTTAAGGGCGAATTC

5 OLIGO start len tm gc% any 3' seq
 LEFT PRIMER 34 20 59.87 50.00 2.00 0.00
 GGGAGATGGAAAAGGAAAGG 233 20 59.23 55.00 2.00 0.00
 RIGHT PRIMER 233 20 59.23 55.00 2.00 0.00
 GACCTCTTCTCACCTCCAA
 SEQUENCE SIZE: 288
 PRODUCT SIZE: 200

10 **OA10-1-A (SEQ ID NO. 183) NOVEL**

15 GAATTCGCCCTTGTGATCGCAGTATTCCTTGTATGGAAGTCATCAGATATGCTGTGCAAGTCTTGCTTAATGTAT
 CTAAGTATGAGAAAACCTTACAGCAGTTTATGATGTAGAAAATTGTATAGATATACTATTGGAGCTTTTGCAGA
 TATGCCGAGAAAAGCCTGGTAATAAAGTTGCAGACAAAGGCGGAAGCATTTTACAAAACTTGTGTTTGTGG
 CTATTTTACTGAAGACAACAATAAAGTGAATACTGAAAGAATACTTTACAAGCAAAAGAAGAATTCTCTA
 TCTACAACTTACAGCTCATAAACATAAAATGAATACTGAAAGAATACTTTACAAGCAAAAGAAGAATTCTCTA
 TAAGCATTCCTTTTATCCCAGAAACACCTGTAAGGACCAGAAATAGTTTCAAGACTTAAGCCAGATTGGGTTTGA
 GAAGAGATAACATGGAAGAAATCACAAATCCCTGCAAGCTATTCAAATGGTGTGGATACGCTTGGCATTCCCTT
 ATTAGTAAATGTAACATTTTTCAGTATGTATAGTGNAAAAGAAATATTAAAGCCAATCATGAGTACGTAACAAAAA
 AAGCTTAAGGGCGAATTC

20 OLIGO start len tm gc% any 3' seq
 LEFT PRIMER 187 20 59.72 40.00 3.00 2.00
 AAGGCGAAGCATTTTACAA 489 20 59.93 45.00 4.00 1.00
 RIGHT PRIMER 489 20 59.93 45.00 4.00 1.00
 CTTGCAGGGGATTGTGATT
 SEQUENCE SIZE: 618
 PRODUCT SIZE: 303

Example 6

Further Selection of Characteristic RNA

30 Those mRNAs exhibiting differential expression following the reverse Northern screening are chosen for further detailed analysis using clinically relevant tissue and secondary reverse Northern analysis. Individual vector-bound cDNA inserts identified, subcloned and sequenced from the initial screen are linearized with an appropriate restriction enzyme and
 35 immobilized on each of six nylon membranes, as described above. The prepared membranes are individually hybridized with radiolabeled probes prepared by reverse transcription of 10 ug total RNA from each of three normal brain tissue or three glioblastoma brain tissue samples. Following reverse transcription, the probes are treated with 20 ug RNase A for 30 minutes at 37°C and purified by Sephadex G50 chromatography. Equivalent amounts of radiolabeled probe
 40 (1.1-1.2 x 10⁶ cpm/ml) are added to the respective blots and hybridized overnight at 57°C. Blots are washed in 2X SSC/1%SDS at 57°C for 30 minutes and analyzed by Phosphor Imaging for 48 hours.

Individual radioactive signals on the blots are quantitated using BioRad Model GS-250 Molecular Imager(R) System and Molecular Analyst(TM)/Macintosh Image Analysis Software

(Version 2.1). One-dimensional profiles are optimized by subtracting image background, as well as pGEM(R) vector control value. An independent Student's t-test is performed comparing the peak heights (in counts) of the three glioblastoma blots, and the three normal brain tissue blots, for each differentially expressed cDNA using SigmaPlot 5.0.

5

Example 7

Isolation of cDNA Clones Related to Differentially Expressed Sequences

The probes selected above as characteristic signals can then be used to identify gene sequences by screening human cDNA libraries. For example approximately 2×10^6 independent clones from a lambda-gt-11 oligo(dT)+random primed human fetal cDNA library (Clontech, Palo Alto, CA) are screened with radiolabeled amplicons from a differentially expressed characteristic signals identified above. Positive plaques are purified by additional screening, and the inserts isolated by subcloning into pGEM(R)7zf(-) vector, sequenced and individually utilized in reverse Northern screening of clinical tissues. The isolated and cloned nucleic acid signals corresponding to the expressed genes of SEQ ID NOS. 1-184 identify the characteristic signals of the invention. Known genes, and the complete nucleic acid sequence for such genes can be obtained from the art, and detection probes designed to specifically identify the expression of such genes in biological samples. In particular, once known, one of ordinary skill in the art can readily identify and prepare hybridization probes which will be suitable for the specific hybridization detection of the desired gene transcript, under a variety of hybridization conditions (*see eg. Molecular Cloning supra*). One of skill in the art is able to select and prepare suitable PCR primers for primer specific amplification of the desired gene transcript. Such primers can be designed to utilize the poly-A tail present on such transcripts, so as to specifically identify transcription products. Inserts identified as novel genes can be further cloned and expanded such that a complete nucleic acid sequence is obtained. However, one of skill in the art will be able to use the nucleic acid sequence of the novel inserts identified in SEQ ID NOS. 1-184, to construct suitable hybridization probes, as well as PCR primers for use in specifically identifying transcripts corresponding to the novel gene represented by the insert.

The characteristic signals listed in SEQ ID NOS. 1-184 are not limited to just these signals, as other further characterizing gene transcripts may also be identified and detected in addition to any one or more of the characteristic signals identified in SEQ ID NOS. 1-184.

Example 8***Kit and Screening Assay for Characteristic Nucleic Acids***

The characteristic diagnostic signal probes, being selected and identified above, are readily adaptable for use in production of screening assay kits. Such kits can include pre-packaged nucleic acid probes corresponding to at least a fragment of the above identified panel sequences, wherein when the assay kit is designed for hybridization detection, such probes are preferably from 10 to 25 nucleic acids in length.

Diagnostic/detection kits designed for use in hybridization and/or PCR based detection of signals can include appropriate paired primers that are specific for the nucleic acid sequences of the characteristic signals identified above, wherein said primers can be preferably 10 to 20 nucleic acids in length, or as suitable for use in automated detection apparatus. One of ordinary skill in the art would be able to design appropriate probes and PCR primers for the selective identification of the specific characteristic signals as listed in SEQ ID NOS. 1-184, and using corresponding modified nucleic acids as desired. One of skill in the art will be further able to design specific PCR primers which will allow for the identification of actively transcribed genes by using the poly-A tail of such transcripts as a primer target, or as a partially anchored primer target. One of ordinary skill in the art would be able to generate suitable primers, and select appropriate amplification conditions and schemes to practice the present invention, and make modifications thereto. (See for example McPherson et al., PCR Volume 1, Oxford University Press, (1991)).

The detection kits of the invention also provide for sets of primers or hybridization probes which can be used to detect specific nucleic acid signals corresponding to one or more of the characteristic signals identified in SEQ ID NOS. 1-184, where such primers or probes are designed to be used in individual reactions, sequential reactions, or combination reaction, using one or more of the primers or probes in the same reaction mixture.

The diagnostic kits of the invention can further encompass suitable buffers for rehydration of dried probes, or dilution of concentrated probe solutions, or for preparing test samples, as needed to accomplish the designated assays. Diagnostic kits can be further designed to provide only the specific primers needed for PCR amplification and detection of the specific signals.

Detection assays, and the kits incorporating such assays of the invention, need not

provide detection of the entire panel of signals, but may be designed to provide for less than the entire nine signals. The assays and kits can incorporate appropriate positive and negative controls, such as the tublin gene, where such control is proliferation dependent, or proliferation independent in signal production. The assay probes designed for PCR can incorporate the appropriate reaction controls, where the absence of such a signal is an indication that said amplification assay physically failed.

Example 8

Screening and Selection of Anti-Cancer Drugs

Using cell cultures of brain cancer cells, or even individual cancer cells, selection of promising drug candidates, and the evaluation of efficacy of various anti-cancer drugs for treating such cancer can be performed in the laboratory, either manually or using automated apparatus. For example, glioblastoma cells, as described above can be administered various doses of anti-cancer drugs, and screened for expression of specific nucleic acid messages corresponding to the panel shown in SEQ ID NOS. 1-184. Any changes in the expression, or expression levels of any species of nucleic acid from this panel, as compared with normal or control cancer cells, would indicate potential for the therapeutic.

Typical anti-cancer drugs which can be specifically screened include Cytarabine, Fludarabine, 5-Fluorouracil, 6-Mercaptopurine, Methotrexate, 6-Thioguanine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Idarubicin, Plicamycin, Carmustine, Iomustine, Cyclophosphamide, Ifosfamide, Mechloroethamine, Streptozotocin, Navelbine, Paclitaxel, Vinblastine, Vincristine, Asparaginase, Cisplatin, Carboplatin, Etoposide, Interferons, Procarbazine.

In addition, various sub-types of brain cancer tissues can be screened for their susceptibility to various anti-cancer therapies, by monitoring any change in the characteristic pattern of expressed genes selected from SEQ ID NOS. 1-184, or fragments or complements thereof, as compared with non-malignant cell expression.

Using the present invention, not only can drug candidates can be screened for potential efficacy using standardized malignant cell cultures, biopsy cells may be cultured and used to screen for efficacy as well. While it would be useful to have long term stable cultures of biopsy cells, the assays of the invention can be performed over a short period of time, thus avoiding the

necessity of long term cultures. Thus, the assay of the invention can be performed on specific brain cancer tissue from individual patients, and the potential efficacy of various therapeutics may be tested on those specific cells.

5 Even if the biopsy sample is not robust enough, or large enough for direct assays of the invention, analysis of the biopsy sample for the characteristic expression of signals, will allow for the selection of a model cancer cell line, which expresses a similar panel of characteristic signals as the biopsy sample. This selected model cell line, and results of therapeutics on the model cell line, may then be used to assess potential therapeutics and treatment.

Example 9

Antisense Inhibition of Gene Expression

10 The invention encompasses antisense therapeutics which can be used to alter gene expression or RNA translation in targeted cells. Antisense therapy can be accomplished using the identified characteristic nucleic acid insert sequences and genes containing the sequence, the entire gene identified as being characteristic, identified known genes, and suitable fragments of
15 all of these nucleic acids. The design and use of antisense therapeutics is described in the art (see for example Eguchi et al., "Antisense RNA", Ann. Rev. Biochem., 1991, 60:631-52). Even more useful than just the insert fragments, the complete nucleic acid sequence for a novel gene, such as CINN-1, and known genes, allows for the preparation of many more anti-sense nucleic acid
20 therapeutics designed for inhibiting translation of the corresponding protein. All antisense nucleic acids can further incorporate modified backbone structures which give unique functionality to the nucleic acid for use as a therapeutic agent. (See for example Verma & Eckstein, (1998), Ann. Rev. Biochem., 67:99-134).

25 For example, antisense nucleic acids, either RNA, DNA or PNAs (Protein nucleic acids) can be designed to be complementary for the nucleic acid sequences given as SEQ ID NOS. 1-184, in their entirety, or a selected fragment thereof. In particular, fragments of from 10 to 15 nucleic acids can be designed based on the sequences of the nucleic acids described by SEQ ID NOS. 1-184. An exemplary antisense molecule from which a 10-15-mer oligo may be selected
30 is SEQ ID NO. 184. Smaller or larger fragments may also be designed, however selection for hybridization strength, and half-life duration in use will need to be made using standard criteria of analysis and established practice in the art.

While a preferred form of the invention has been shown in the drawings and described, since variations in the preferred form will be apparent to those skilled in the art, the invention should not be construed as limited to the specific form shown and described, but instead is as set
5 forth in the claims.

CLAIMS

WE CLAIM:

1. A method for ascertaining the propensity of a cell for malignant phenotype said cell being isolated or in a biological sample, said method comprising assaying a cell or biological sample to be tested for a signal indicating the transcription of a nucleic acid transcript, wherein said transcript is from at least one gene selected from the group consisting of nucleic acid sequences identified in SEQ ID NOS. 1-184.
2. A method of claim 1 wherein said nucleic acid is selected from the group consisting of SEQ ID NO. 68, 69, 142, 143, 144, 147, 149, 162, 173, and 183.
3. A method of claim 1 wherein said nucleic acid is selected from the group consisting of SEQ ID NO. 68, 69 and 183.
4. The method of claim 1, wherein said expressed gene is detected by RT-PCR using at least one gene specific amplification primer.
5. The method of claim 1, wherein said expressed gene is detected by nucleic acid hybridization using at least one gene specific probe.
6. The method of claim 5, wherein said assay is *in situ* hybridization.
7. The method of claim 1, wherein a protein encoded by said expressed gene is detected by protein gel assay.
8. The method of claim 1, wherein a protein encoded for by said expressed gene is detected by antibody binding assay.
9. The method of claim 1, wherein said expressed gene is detected by RNase protection assay.
10. The method of claim 1, wherein said gene contains a nucleic acid sequence corresponding to a portion of the nucleic acid sequence selected from the group consisting of SEQ ID NOS. 1-184.
11. A method for ascertaining the suitability of an anti-neoplastic drug candidate for efficacy in treating a malignancy, wherein said method comprises combining said candidate drug with a cell having a cancer phenotype, said cell being isolated or in a biological sample, detecting in said cell or biological sample any change in the expression of at least one of the genes selected

from the group consisting of the nucleic acid sequences of SEQ

12. A method of claim 1 wherein said nucleic acid is selected from the group consisting of SEQ ID NO. 68, 69, 142, 143, 144, 147, 149, 162, 173, and 183.

13. A method of claim 1 wherein said nucleic acid is selected from the group consisting of SEQ ID NO. 68, 69 and 183.

14. A method of claim 11, wherein said malignant biological sample is a biopsy sample from a patient to be treated.

15. A method as in claim 14, wherein said malignant biological sample is a cell line.

16. A method as in claim 14, wherein said malignant biological sample is a cell.

17. A therapeutic compound identified in the method of claim 11.

18. A kit comprising hybridization probes specific for at least two nucleic acid sequences selected from the group consisting of the characteristic nucleic acid sequences identified in SEQ ID NOS. 1-184.

19. A kit of claim 18 wherein said nucleic acid is selected from the group consisting of SEQ ID NO. 68, 69, 142, 143, 144, 147, 149, 162, 173, and 183.

20. A kit of claim 18 wherein said nucleic acid is selected from the group consisting of SEQ ID NO. 68, 69 and 183.

21. A kit of claim 18, further comprising suitable reaction buffer components.

22. A kit of claim 18, wherein said probes are suitable for use in PCR amplification of the specific target.

23. A kit of claim 18, wherein said probes are suitable for *in situ* hybridization.

24. A kit comprising probes specific for at least one protein containing an amino acid sequence corresponding to the translation of at least one nucleic acid sequence selected from the group consisting of SEQ ID NOS. 1-184.

25. A kit as in claim 24, where said probe is an antibody, or antigen binding fragment thereof.

26. A kit as in claim 25, where said probe is a polyclonal antibody.

27. A kit as in claim 25, where said probe is a monoclonal antibody.

28. An isolated nucleic acid comprising a nucleic acid sequence selected from the group

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OS. 1-184.

PCT/US00/31809

expression vector comprising a nucleic acid sequence of claim 28.

consist of:
A transformed host cell comprising a nucleic acid sequence of claim 28 operably
a transcription regulation component.

FIGURE 1

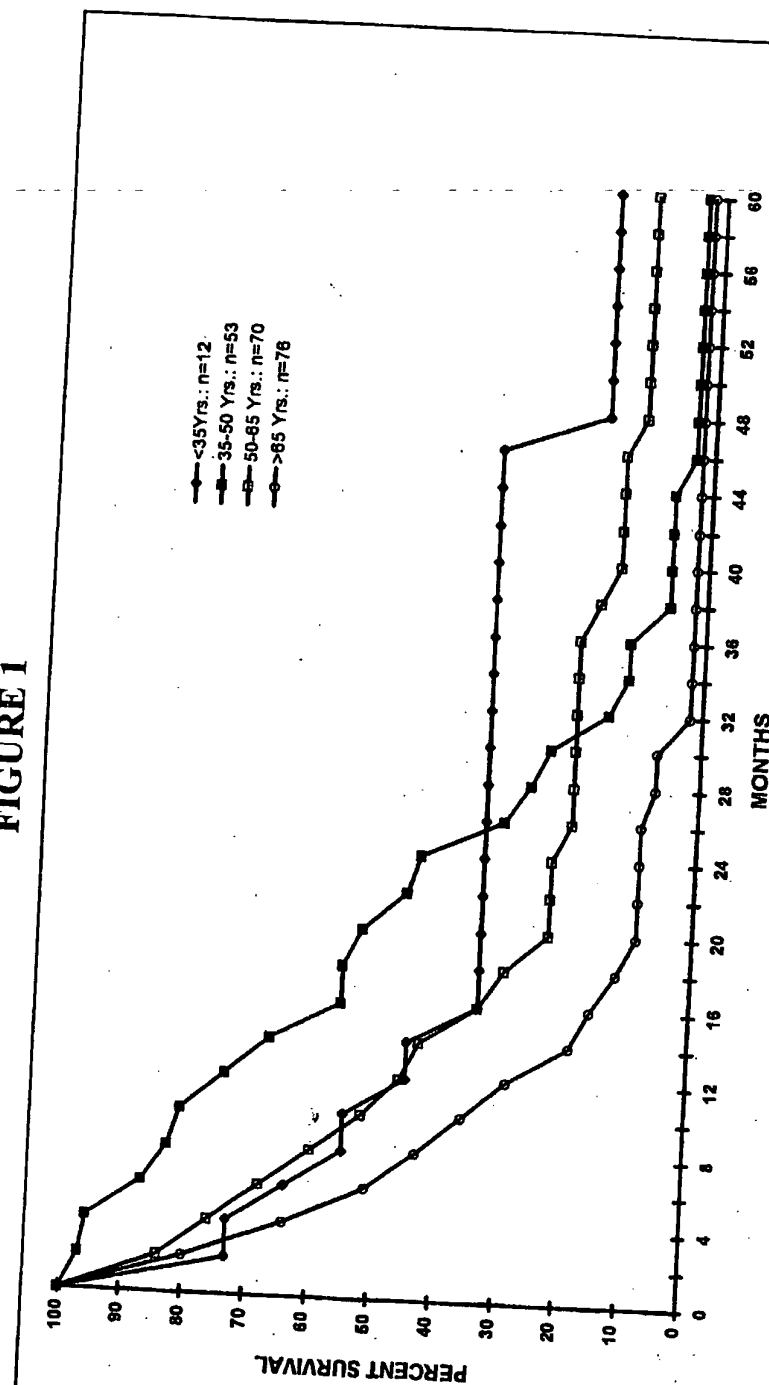


Figure 1. Relationship between patient age at diagnosis and glioma survival.
 The survival pattern for Grade IV astrocytoma (GBM) patients according to four age strata is illustrated. The apparent differences between the <35 and the $35-50$ year group are not statistically significant, but the survival for the <50 year group as a whole was statistically different from the $50-65$ and the >65 year groups (Wilcoxon test, $p=.002$).

FIGURE 2

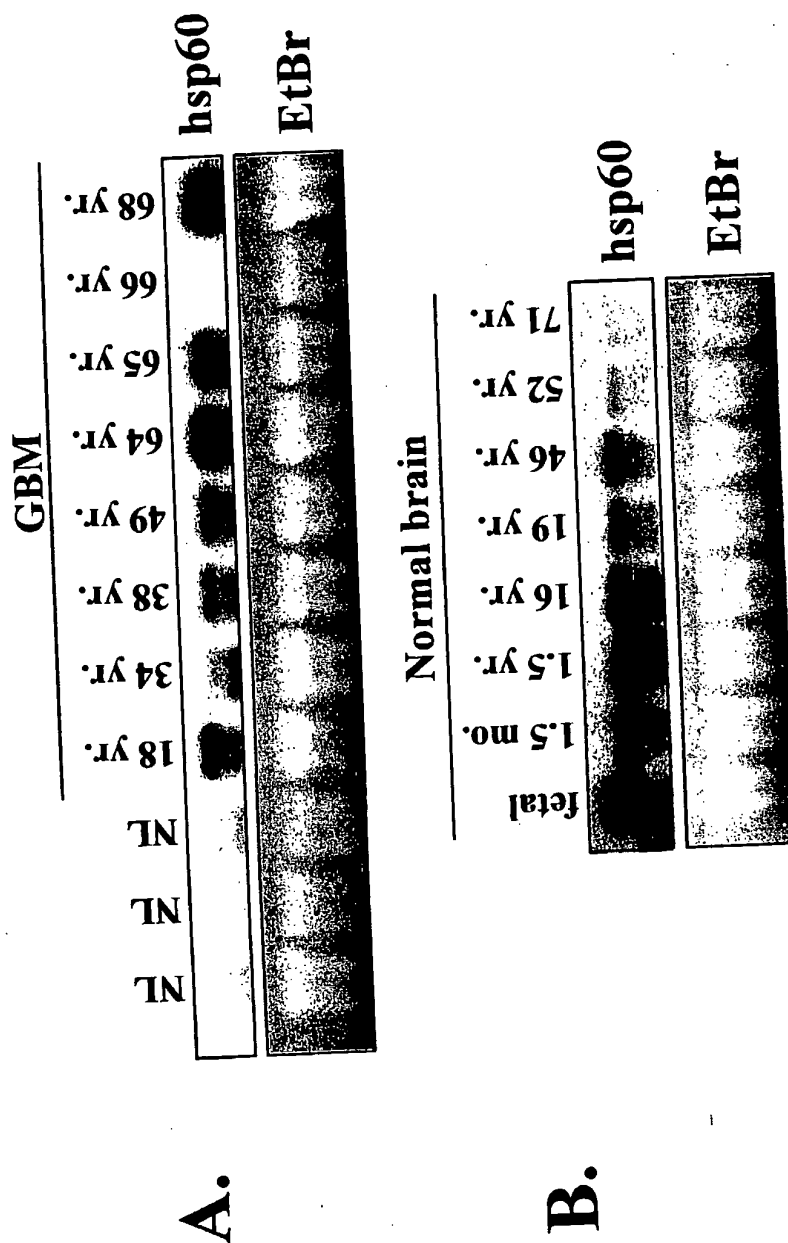


Figure 2. Northern analysis of hsp60 mRNA. 25µg of total RNA was isolated, electrophoresed through 1.2% agarose-formaldehyde gels, transferred to nylon membranes and hybridized with a uniformly [³²P]-labeled hsp60-specific cDNA probe. (A): hsp60 expression in normal (NL) brain and GBMs. Patient age at diagnosis is depicted. (B): Developmental expression of hsp60 in normal brain tissue.

FIGURE 3

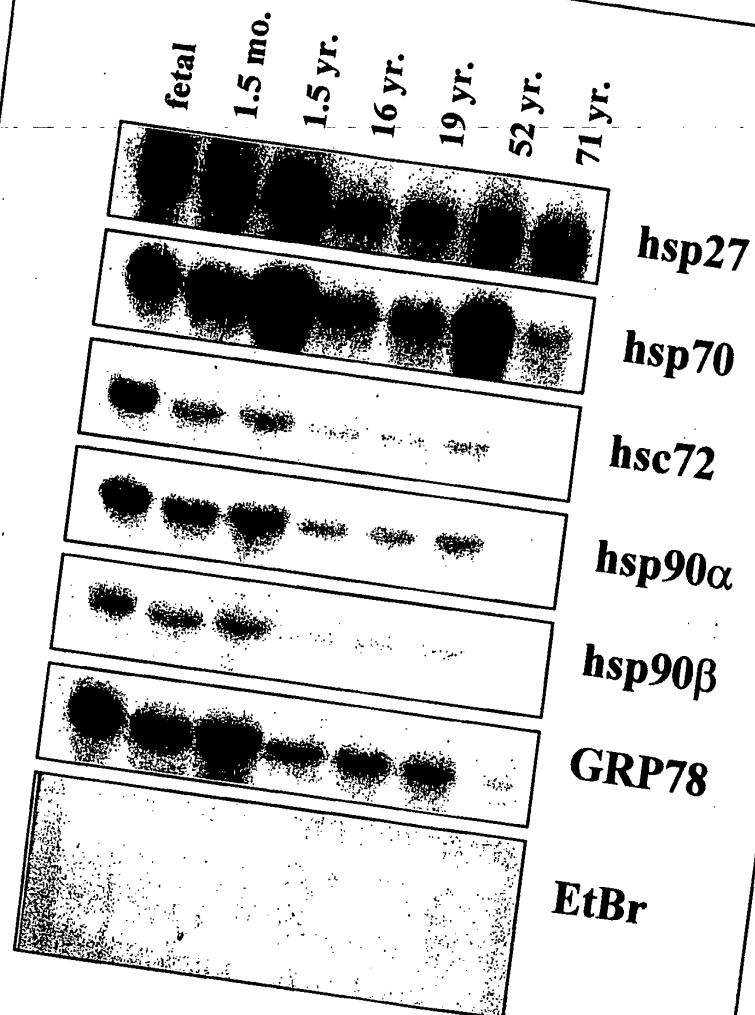


Figure 3. Normal Developmental Expression of Heat Shock Proteins in Human Brain. 25 μ g of total RNA was isolated, electrophoresed through 1.2% agarose-formaldehyde gels, transferred to nylon membranes and hybridized with a uniformly [32 P]-labeled cDNA probes specific for hsp27, hsp70, hsc72, hsp90 α , hsp90 β , and GRP78.

FIGURE 4

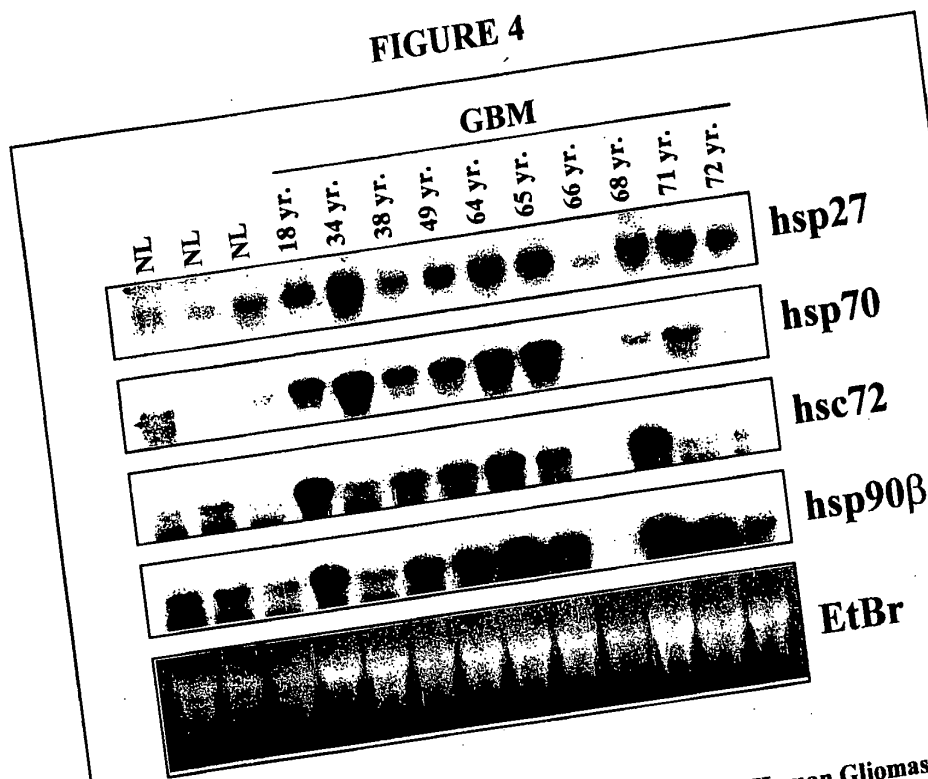


Figure 4. Differential Expression of Heat Shock Proteins in Human Gliomas. 25 μ g of total RNA was isolated, electrophoresed through 1.2% agarose-formaldehyde gels, transferred to nylon membranes and hybridized with a uniformly [32 P]-labeled cDNA probes specific for hsp27, hsp70, hsc72, and hsp90 β .